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THE PROTECTION OF THE OVULES IN FLOWERING PLANTS

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INTRODUCTION

The connecting link between successive generations is, in all multicellular and sexual organisms, a defenseless embryo. Special means for the protection of this vital stage may be recognized in most higher organisms. The establishment of conditions of increased security for the embryo and immature young is indeed a dominant motif in the progressive evolution of both the vertebrates and the insects. The ovule of the seed plants represents in itself a relatively advanced stage in the protection of the embryo, which is here retained within a spore wall, a sporangium, and a pair of fused subtending stems (the integuments, Florin, 1939, 1944, pp. 651–653). In the most advanced and successful gymnosperms, the pines and araucarias, the ovule is fused onto the surface of a scale and encased within a heavily armed cone. This trend has been carried still further in the angiosperms with the enclosure of the ovule within the folded foliar organ upon which it is borne. In most angiosperms the carpels are themselves fused together and the ovules borne on placentae in the center of the compound ovary. In many angiosperms, finally, the ovary is buried beneath the other organs of the flower.

The indication that the changes in the position of the ovules in higher plants are part of a progressive and long-continued trend constitutes presumptive evidence that those changes have been guided by natural selection. No other explanation of the phenomenon of progressive evolution has, at any rate, ever been verified. It follows that a study of the position of the ovules in various groups of plants, as correlated with special conditions in their life histories, should throw light upon the adaptive value of many structural arrangements associated with the position of the ovules. The purpose of the present paper is to inquire into the relation between the characteristics of the animals which visit the flowers of angiosperms and the occurrence of special means of ovule protection. The evidence to be presented suggests that flowers which are probed by the bill of a bird or the chewing jaws of a beetle have evolved more advanced means of ovule protection than flowers which are sucked by the slender proboscis of a bee, wasp, fly, moth or butterfly.

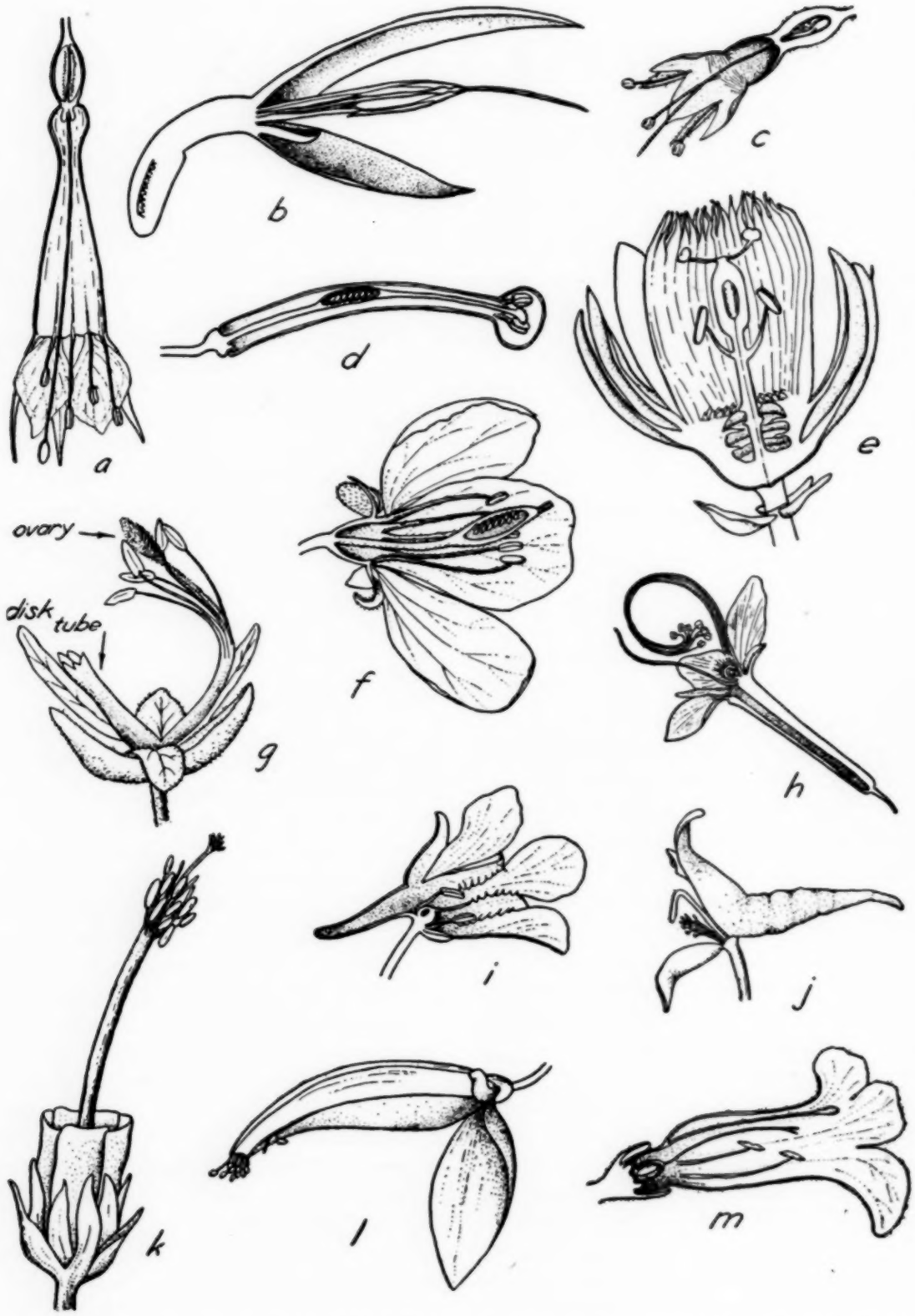

FIG. 1. Longisectional views of bird flowers showing various means of ovule protection: inferior ovary (a–c); stiped ovary (d–g); spur (h–j); stamen column (k–l); no special protection (m).

a—*Fuchsia fulgens* (Onagraceae); b—*Strelitzia augusta* (Strelitziaceae); c—*Combretum grandiflorum* (Combretaceae); d—*Embothrium grandiflorum* (Proteaceae); e—*Passiflora alata* (Passifloraceae); f—*Melanoxylon brauna* (Caesalpiniaceae); g—*Cadaba farinosa* (Cap-

Ovule Protection in Bird Flowers

Birds commonly injure flowers in feeding upon them. The Bullfinch thus bites off the corolla and eats the ovules and nectar of *Primula* in England (Darwin, 1874). Hummingbirds damage the style of *Odontadenia* in Brazil (Malme, 1923). The white-eye *Zosterops* bites off the stamens of *Prunus* and *Camellia* in Japan (Molisch, 1926), the Drongo (*Dicrurus*) tears off the stamens of *Erythrina* in Java (Porsch, 1924), and the Lory eats the stamens of *Eugenia* in East Africa (Stresemann, 1914). Hummingbirds puncture the floral tube of *Fuchsia* and *Salvia* in South America and of *Lonicera* in North America (Knuth and Loew, 1904–1905; Epling, comm.). The Sunbird (*Cinnyris*) splits open the flowers of *Loranthus* in pollinating them in Natal (Evans, 1895), and similarly slits the corollas of *Kniphofia, Canna, Dracaena* and *Gardenia* in Rhodesia (Swynnerton, 1915a). The Warbler (*Sylvia*) eats the calyx of *Hibiscus* and *Abutilon* and the Willow Warbler (*Phylloscopus*) eats the corolla of *Aloe* in North Africa (Lowe, 1896). The Titmouse (*Parus*) damages flowers of *Ribes* in probing for nectar in Ireland (Swynnerton, 1915b), and Baltimore Orioles injure the flowers of *Ribes* in North America (Porsch, 1924). Darwin's Finch (*Geospiza*) destroys cactus flowers on the Galapagos Islands (Marshall, 1920). The spiny-cheeked Honeyeater (*Acanthagenys*) injures the flowers of *Correa* in Australia (Porsch, 1927b).

The destructive habits of birds in their visits to flowers may be associated with the presence of strengthening tissues in many bird flowers. The strong tissues of which the flowers of *Erythrina* are constructed may be cited as a typical example. The ecological significance of these mechanical tissues is indicated by the fact that in Rhodesia the flowers of *Erythrina* suffer less damage from the probing bills of Sunbirds than the flowers of some other plants, such as *Dracaena* and *Gardenia* (Swynnerton, 1915a). The strength of many bird flowers is due to the development of tissues not generally found in flowers pollinated by long-tongued insects. The flowers of *Eucalyptus*, for example, are built of hard sclerenchymatous tissues. Sclereids are abundant in both the receptacle and the style (Porsch, 1927a). *Castanospermum australe* is histologically quite different from *Eucalyptus* in that the mechanical tissue here is collenchyma and is present in the corolla (*loc. cit.*). *Adenanthos obovata* is marked by the development of nests of sclereids in the corolla and tracheary elements of the style (Porsch, 1927b). Several Australian species of *Loranthus* investigated by Porsch proved to have different histological features leading to a mechanical strengthening of the flowers. The perianth of *Loranthus pendulus* is strengthened by collenchyma, that of *L. linophyllus* by sclereids distributed throughout its whole length; in *L. sanguineus* almost the entire inner part of the corolla is composed of sclereids, whereas in *L. quandang* the sclereids are grouped on the outer face of the corolla, and in *L. celastroides* it is the tip of the corolla that is woody (Porsch, 1929). In the Hawaiian palm *Pritchardia* sclereids are developed widely in the perianth (Porsch, 1930).

The protection of the ovules in bird flowers, which is a far more vital matter than the strengthening of the perianth, appears to be accomplished in several quite different ways. The various con-

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paridaceae); h—*Acioa guianensis* (Rosaceae); i—*Tropaeolum moritzianum*,(Tropaeolaceae); j—*Delphinium scopulorum* (Ranunculaceae); k—*Dombeya cannabina* (Sterculiaceae); l—*Erythrina crista-galli* (Papilionaceae); m—*Jacaranda tomentosa* (Bignoniaceae).

Illustrations redrawn from Engler and Prantl, except (j) which is original. The flowers are shown as nearly as possible in their natural positions; the flower of *Fuchsia* is pendant, that of *Erythrina* inverted.

trivances fulfil, however, one fundamental condition, namely the spatial separation of the nectary and the ovary. The probing bill of the bird is thereby separated from the delicate ovules which it is the role of the bird to pollinate. This separation may come about in either the vertical or the horizontal direction. Vertically, the ovary may occupy a position beneath the nectary (fig. 1, a–c) or it may be elevated on a stipe above the nectary (d–g). Horizontally, a sheath composed of the fused bases of the stamens may be interposed between the ovary and the nectar (k–l), or the nectar may be stored in a spur which passes to one side of the ovary (h–j). That these modifications of the original form of the flower do in fact guard the ovules from the bill of the birds is evidenced by the observations of the author. In the pages which follow the foregoing structural modifications of the flower will, for convenience, be discussed as if they were means of ovule protection; the question of their actual adaptive value will be reserved for a final discussion.

The distribution of the foregoing and other structural features in bird flowers has been studied in the following way. A list was compiled of all of the angiospermous genera which contain species known to be visited by birds for nectar or pollen. The birds considered as especially important for the purpose of this list are members of the Nectariniidae, Zosteropidae and Dicaeidae in the Paleotropics, the Meliphagidae and Trichoglossidae in Australia, the Drepanididae in Hawaii, and the Trochilidae and Coerebidae in North and South America. The writings of Porsch formed the principal source for the list (Porsch, 1924, 1926, 1927a, 1927b, 1929a, 1929b, 1930); additional data were obtained from Knuth and Loew (1904), Melin (1935), Pickens (1936, 1941), Bené (1946), Wagner (1946), James (1948), and unpublished observations of the author. The generic names were brought up to date with the aid of Willis (1931) and Index Kewensis; segregate genera were combined wherever possible. The genera were then arranged into families and orders in accordance with the system of Hutchinson (1924–1936). The presence or absence of any special morphological characters in the flowers of these genera that might be construed as means of ovule protection was next recorded on the basis of the descriptions and illustrations in Engler and Prantl (1888–1898). The annotated list of bird flowers is presented here as part A of table 1.

TABLE 1. *The genera of flowering plants which are known to be visited by various animals for nectar or pollen, grouped according to probable agent of pollination, and annotated according to the presence of special means of ovule protection*

(Abbreviations: e = epigyny; p = perigyny; stam = stamen column; stip = stiped ovary; no = no special means of ovule protection. For further explanation see text.)

A—BIRD FLOWERS

RANALES. *Ranunculaceae.*—Aquilegia (spur); Delphinium (spur). CAPPARIDALES. *Capparidaceae.*—Capparis (stip); Cleome (stip); Crataeva (stip); Gynandropsis (stip). *Moringaceae.*—Moringa (stip). VIOLALES. *Violaceae.*—Calyptrion (spur); Paypayrola (stam). POLYGALALES. *Vochysiaceae.*—Erisma (spur); Qualea (spur); Salvertia (spur); Vochysia (spur). SAXIFRAGALES. *Crassulaceae.*—Bryophyllum (no); Cotyledon (no); Kalanchoe (no); Rochea (no). CARYOPHYLLALES. *Caryophyllaceae.*—Saponaria (no). CHENOPODIALES. *Amarantaceae.*—Gomphrena (stam). GERANIALES. *Geraniaceae.*—Pelargonium (spur). *Tropaeolaceae.*—Tropaeolum (spur). *Balsaminaceae.*—Impatiens (spur). LYTHRALES. *Lythraceae.*—Cuphea (spur); Grislea (no); Lafoensia (no); Woodfordia (no). *Sonneratiaceae.*—Duabanga (no); Sonneratia (no). *Onagraceae.*—Epilobium (e); Fuchsia (e); Oenothera (e); Zauschneria (e). THYMELAEALES. *Penaeaceae.*—Sarcocolla (no). *Nyctaginaceae.*—Bougainvillea (stam). PROTEALES. *Proteaceae.*—Adenanthos (no); Banksia (no); Darlingia (no); Dryandra (no); Embothrium (stip); Euplassa (ovary armed); Grevillea (stip); Hakea (stip); Isopogon (no); Knightia (no); Lambertia (ovary armed); Leucospermum (no); Mimetes (no); Protea (no); Panopsis (no); Roupala (stip); Telopea (stip). PITTOSPORALES. *Pittosporaceae.*—Pittosporum

TABLE 1.—*Continued*

(no). BIXALES. *Cochlospermaceae.*—Cochlospermum (no). TAMARICALES. *Fouquieriaceae.*—Fouquiera (no). PASSIFLORALES. *Passifloraceae.*—Dilkea (stip); Passiflora (stip). CUCURBITALES. *Cucurbitaceae.*—Cayaponia (e); Gurania (e). *Begoniaceae.*—Begonia (e). *Caricaceae.*—Carica (no); Jacaratia (no). CACTALES. *Cactaceae.*—Cereus (e); Chamaecereus (e); Echinocactus (e); Epiphyllum (e); Melocactus (e); Nopalea (e); Opuntia (e); Zygocactus (e). THEALES. *Theaceae.*—Bonnetia (no). *Marcgraviaceae.*—Norantea (spur); Marcgravia (spur); Souroubea (spur). *Caryocaraceae.*—Caryocar (no). MYRTALES. *Myrtaceae.*—Angophora (e); Beaufortia (e); Callistemon (e); Calothamnus (e); Darwinia (e); Eucalyptus (e); Eugenia (e); Feijoa (e); Leptospermum (e); Melaleuca (e); Metrosideros (e); Myrrhinium (e); Psidium (e); Tristania (e). *Lecythidaceae.*—Barringtonia (e); Couroupita (e); Planchonia (e). *Melastomaceae.*—Axinaea (e); Bellucia (e); Blakea (e); Brachyotum (no); Henriettea (e); Lavoisiera (e); Miconia (e); Mouriria (e); Tibouchina (e); Tococa (e). *Combretaceae.*—Combretum (e); Lumnitzera (e). *Rhizophoraceae.*—Bruguiera (e); Rhizophora (e). GUTTIFERALES. *Guttiferae.*—Clusia (no); Kielmeyera (no); Moronobea (stam); Platonia (stam); Symphonia (stam); Vismia (no). TILIALES. *Tiliaceae.*—Luehia (no); Elaeocarpus (stam); Mollia (no). *Sterculiaceae.*—Brachychiton (stam); Chiranthodendron (stam); Helicteres (stam); Pterospermum (stam); Theobroma (stam). *Bombacaceae.*—Bombax (stam); Cavanillesia (stam); Ceiba (stam); Chorisia (stam); Durio (stam); Matisia (stam); Quararibea (stam); Scleronema (stam). MALVALES. *Malvaceae.*—Abutilon (stam); Cienfuegosia (stam); Goethea (stam); Gossypium (stam); Hibiscadelphus (stam); Hibiscus (stam); Malvaviscus (stam); Nototriche (stam); Pavonia (stam); Thespesia (stam). EUPHORBIALES. *Euphorbiaceae.*—Euphorbia (stip); Manihot (no); Pedilanthus (stip). CUNONIALES. *Escalloniaceae.*—Escallonia (e). *Grossulariaceae.*—Ribes (e). ROSALES. *Rosaceae.*—Couepia (spur); Cydonia (e); Eriobotrya (e); Prunus (p); Pyrus (e). LEGUMINOSAE. *Caesalpiniaceae.*—Aldina (stip); Amherstia (stip); Apalatoa (stip); Bauhinia (stip); Brownea (stip); Caesalpinia (stip); Cassia (stip); Dicymbe (stip); Elizabetha (stip); Eperua (stip); Exostyles (stip); Heterostemon (stip); Hymenaea (stip); Mezoneurum (stip); Parkinsonia (stip); Phyllocarpus (stip); Poinciana (stip); Swartzia (stip); Tamarindus (stip + spur). *Mimosaceae.*—Acacia (stam or stip); Affonsea (stam); Albizzia (stam); Calliandra (stam); Dichrostachys (no); Elephantorrhiza (no); Entada (no); Inga (stam); Mimosa (stip); Neptunia (stip); Parkia (stam or stip); Pentaclethra (stam); Piptadenia (no); Pithecolobium (stam); Plathymenia (stip); Prosopis (stip); Stryphnodendron (stip). *Papilionaceae.*—Barbiera (stam); Brachysema (stip); Butea (stam); Camptosema (stam); Canavalia (stam); Castanospermum (stip); Centrosema (stam); Clianthus (stam); Clitoria (stam); Coublandia (stam); Crotalaria (stam); Erythrina (stam); Galactia (stam); Gourliea (stip); Harpalyce (stam); Kennedya (stam); Lupinus (stam); Mucuna (stam); Periandra (stam); Robinia (stam); Sesbania (stam); Sophora (stip); Strongylodon (stam); Sutherlandia (stam). OLACALES. *Olacaceae.*—Chaunochiton (e). SANTALALES. *Loranthaceae.*—Aetanthus (e); Loranthus (e); Nuytsia (e); Phrygilanthus (e); Psittacanthus (e). *Santalaceae.*—Santalum (e). RUTALES. *Rutaceae.*—Citrus (no); Cusparia (p); Decagonocarpus (p); Erythrochiton (p); Platydesma (no); Raputia (p); Ravenia (p); Spiranthera (no). *Simarubaceae.*—Quassia (no); Simaba (stip). SAPINDALES. Sapindaceae.—Aesculus (no); Serjania (no). *Melianthaceae.*—Melianthus (spur). *Anacardiaceae.*—Anacardium (no). UMBELLIFLORAE. *Araliaceae.*—Sciadophyllum (e); Tetraplasandra (e). ERICALES. *Ericaceae.*—Erica (no); Gaultheria (no); Leucothoe (no); Rhododendron (no); Sarcodes (no). *Vacciniaceae.*—Gaylussacia (e); Psammisia (e); Thibaudia (e); Vaccinium (e). *Epacridaceae.*—Epacris (no); Styphelia (no). EBENALES. *Ebenaceae.*—Diospyros (no). MYRSINALES. *Myrsinaceae.*—Aegiceras (no). STYRACALES. *Styracaceae.*—Styrax (stam). *Simplococaceae.*—Symplocos (e). *Diclidantheraceae.*—Diclidanthera (no). LOGANIALES. *Loganiaceae.*—Antonia (no); Buddleia (no); Desfontainea (no); Fagraea (no); Labordia (no); Spigelia (no); Strychnos (no). APOCYNALES. *Apocynaceae.*—Allamanda (no); Dipladenia (no); Echites (no); Laseguea (no); Mandevilla (no); Nerium (no); Odontadenia (no); Plumeria (no); Prestonia (no); Rauwolfia (no); Rhabdadenia (no); Rhodocalyx (no); Stipecoma (no); Skytanthus (no); Thevetia (no); Tabernaemontana (no); Voacanga (no). *Asclepiadaceae.*—Asclepias (stam). RUBIALES. *Rubiaceae.*—Capirona (e); Cassupa (e); Cephaelis (e); Cinchona (e); Condaminea (e); Coussarea (e); Coutarea (e); Duroia (e); Faramea (e); Ferdinandusa (e); Genipa (e); Guettarda (e); Hamelia (e); Henriquezia (e); Hindsia (e); Hippotis (e); Isertia (e); Ixora (e); Joosia (e); Ladenbergia (e); Macrocnemum (e); Manettia (e); Palicouria (e); Pogonopus (e); Port-

TABLE 1.—*Continued*

landia (e); Psychotria (e); Retiniphyllum (e); Rudgea (e); Rustia (e); Sabicea (e); Sickingia (e); Sphinctanthus (e); Thieleodoxa (e); Ucriana (e); Warscewiczia (e). *Caprifoliaceae.*—Abelia (e); Lonicera (e). ASTERALES. *Compositae.*—Augusta (e); Barnadesia (e); Carduus (e); Centaurea (e); Chuquiragua (e); Cnicothamnus (e); Eremanthus (e); Gochnatia (e); Helianthus (e); Hesperomannia (e); Lychnophora (e); Moquinia (e); Mutisia (e); Piptocarpha (e); Pluchea (e); Raillardia (e); Seris (e); Trichocline (e); Trixis (e); Vernonia (e); Wunderlichia (e); Zinnia (e). GENTIANALES. *Gentianaceae.*—Lisianthus (no); Prepusa (no); Schultesia (no). CAMPANALES. *Lobeliaceae.*—Centropogon (e); Clermontia (e); Cyanea (e); Delissea (e); Lobelia (e); Rollandia (e); Siphocampylus (e); Trematolobelia (e). *Goodeniaceae.*—Scaevola (e). POLEMONIALES. *Polemoniaceae.*—Cantua (no); Gilia (no). BORAGINALES. *Boraginaceae.*—Cordia (no); Lobostemon (no). SOLANALES. *Solanaceae.*—Cestrum (no); Datura (no); Iochroma (no); Lycium (no); Metternichia (no); Nicotiana (no); Nothocestrum (no); Petunia (no); Schwenkia (no); Solandra (no). *Convolvulaceae.*—Convolvulus (no); Ipomoea (no); Jacquemontia (no); Maripa (no); Prevostea (no). PERSONALES. *Scrophulariaceae.*—Castilleja (no); Escobedia (no); Esterhazya (no); Gerardia (no); Halleria (no); Lamourouxia (no); Macranthera (no); Mimulus (no); Pedicularis (no); Pentstemon (no); Physocalyx (no); Russelia (no); Wigthia (no). *Gesneriaceae.*—Agalmyla (corolla constricted above ovary); Asteranthera (no); Besleria (no); Corytholoma (e); Crantzia (no); Cyrtandra (no); Dichrotrichum (corolla constricted above ovary); Drymonia (no); Episcia (no); Gesneria (e); Gloxinia (e); Hypocyrta (no); Mitraria (no); Nematanthus (no); Paliavana (e); Sarmienta (no); Sinningia (e); Trichosporum (corolla constricted above ovary); Vanhouttea (e). *Bignoniaceae.*—Adenocalymna (no); Amphilophium (no); Anemopaegma (stip); Arrabidaea (no); Bignonia (no); Callichlamys (no); Colea (no); Crescentia (spur); Cuspidaria (no); Cybistax (no); Distictis (no); Eccremocarpus (no); Fridericia (no); Glaziova (no); Jacaranda (no); Kigelia (no); Lundia (no); Macfadyena (no); Mansoa (no); Martinella (no); Melloa (no); Memora (no); Neojobertia (no); Oroxylon (no); Petastoma (no); Pithecoctenium (no); Pleonotoma (no); Radermachera (no); Saldanhaea (no); Sparattosperma (no); Spathodea (no); Tecoma (no); Tabebuia (no); Zeyheria (stip). *Acanthaceae.*—Aphelandra (no); Beloperone (no); Brachystephanus (no); Clistax (no); Dicliptera (no); Drejera (no); Geissomeria (no); Harpochilus (no); Jacobinia (no); Justicia (no); Lophostachys (no); Mendoncia (no); Odontonema (no); Pachystachys (no); Ruellia (no); Sanchezia (no); Schaueria (no); Staurogyne (no); Stenostephanus (no). LAMIALES. *Myoporaceae.*—Pholidia (no). *Verbenaceae.*—Bouchea (no); Clerodendron (no); Lantana (no); Stachytarpheta (no); Taligalea (no); Verbena (no); Vitex (no); Holmskioldia (no). *Labiatae.*—Coleus (no); Haplostachys (no); Hedeoma (no); Hyptis (no); Leonotis (no); Monarda (no); Nepeta (no); Phyllostegia (no); Salvia (no); Sphacele (no); Stenogyne (no). COMMELINALES. *Commelinaceae.*—Tradescantia (no). BROMELIALES. *Bromeliaceae.*—Aechmea (e); Ananas (e); Billbergia (e); Brocchinia (e); Bromelia (e); Canistrum (e); Caraguata (no); Cryptanthus (e); Dyckia (no); Encholirion (no); Fernseea (e); Gravisia (e); Neoglaciovia (e); Nidularium (e); Pitcairnia (no); Portea (e); Puya (no); Quesnelia (e); Streptocalyx (e); Tillandsia (no); Vriesea (no). ZINGIBERALES. *Musaceae.*—Musa (e). *Strelitziaceae.*—Heliconia (e); Ravenala (e); Strelitzia (e). *Zingiberaceae.*—Costus (e); Elettaria (e); Hedychium (e); Hornstedtia (e); Phaeomeria (e); Renealmia (e); Riedelia (e). *Cannaceae.*—Canna (e). *Marantaceae.*—Calathea (e); Ischnosiphon (e); Stromanthe (e). LILIALES. *Liliaceae.*—Aloe (no); Gloriosa (no); Hemerocallis (no); Kniphofia (no); Lachenalia (no). *Pontederiaceae.*—Eichornia (no). ALSTROEMERIALES. *Alstroemeriaceae.*—Alstroemeria (e); Bomarea (e). *Philesiaceae.*—Philesia (stam); Lapageria (no). AMARYLLIDALES. *Amaryllidaceae.*—Crinum (e); Griffinia (e); Hippeastrum (e); Nerine (e); Pancratium (e). IRIDALES. *Iridaceae.*—Antholyza (e); Cypella (e); Gladiolus (e); Iris (e); Watsonia (e). AGAVALES. *Xanthorrhoeaceae.*—Xanthorrhoea (no). *Agavaceae.*—Agave (e); Dracaena (no); Phormium (no); Yucca (no). PALMALES. *Palmae.*—Cocos (no); Pritchardia (no). PANDANALES. *Pandanaceae.*—Freycinetia (flowers congested in inflorescence). HAEMODORALES. *Haemodoraceae.*—Anigozanthos (e). *Velloziaceae.*—Barbacenia (e); Vellozia (e). ORCHIDALES. *Orchidaceae.*—Angraecum (e + spur); Bifrenaria (e + spur); Cattleya (e); Comparettia (e + spur); Cryptochilus (e); Cymbidium (e); Disa (e + spur); Epidendrum (e); Eulophia (e + spur); Galeandra (e + spur); Laelia (e); Lissochilus (e + spur); Lycaste (e + spur); Masdevallia (e); Pelexia (e + spur); Pogonia (e); Saccolabium (e + spur); Satyrium (e + spur); Sobralia (e); Sophronitis (e); Spiranthes (e); Vanilla (e).

TABLE 1.—*Continued*

B—BAT FLOWERS

LYTHRALES. *Sonneratiaceae.*—Sonneratia (no). CACTALES. *Cactaceae.*—Cereus (e). THEALES. *Caryocaraceae.*—Caryocar (no). MYRTALES. *Myrtaceae.*—Eugenia (e). *Lecythidaceae.*—Barringtonia (e). TILIALES. *Sterculiaceae.*—Dombeya (stam). *Bombacaceae.*—Adansonia (stam); Ceiba (stam); Durio (stam); Ochroma (stam). ROSALES. *Rosaceae.*—Eriobotrya (e). LEGUMINOSAE. *Caesalpiniaceae.*—Bauhinia (stip); Eperua (stip). *Mimosaceae.* Calliandra (stam); Parkia (stam or stip). *Papilionaceae.*—Erythrina (stam). EBENALES. *Sapotaceae.—Bassia* (no); Omphalocarpum (no); Palaquium (no); Payena (no). SOLANALES. *Convolvulaceae.*—Erycibe (no). PERSONALES. *Bignoniaceae.*—Crescentia (spur); Kigelia (no); Markhamia (no); Oroxylon (no); Parmentiera (no). ZINGIBERALES. *Musaceae.*—Musa (e). AGAVALES. *Agavaceae.*—Agave (e). PALMALES. *Palmae.*—Areca (no); Cocos (no). PANDANALES. *Pandanaceae.—Freycinetia* (flowers congested in inflorescence).

C—BEETLE FLOWERS

MAGNOLIALES. *Magnoliaceae.*—Magnolia (no); Paeonia (no). *Winteraceae.*—Illicium (no). ANONALES. *Eupomatiaceae.*—Eupomatia (p). RANALES. *Nymphaeaceae.*—Euryale (p); Nelumbo (p); Nymphaea (p); Victoria (p). RHOEADALES. *Papaveraceae.*—Eschscholtzia (p). LYTHRALES. *Punicaceae.*—Punica (e). ROSALES. *Rosaceae.*—Fragaria (no); Rosa (p); Rubus (no); Spiraea (p). *Calycanthaceae.*—Calycanthus (p). SANTALALES. *Balanophoraceae.*—Helosis (e). RHAMNALES. *Vitaceae.*—Vitis (no). UMBELLIFLORAE. *Cornaceae.*—Cornus (e). *Umbelliferae.*—Aegopodium (e); Angelica (e); Anthriscus (e); Carum (e); Heracleum (e); Myrrhis (e); Oenanthe (e); Pimpinella (e). ERICALES. *Ericaceae.*—Pyrola (no). RUBIALES. *Caprifoliaceae.*—Sambucus (e). ASTERALES. *Dipsacaceae.*—Knautia (e). *Compositae.*—Achillea (e); Adenostyles (e); Chrysanthemum (e). ARALES. *Araceae.*—Arum (flowers congested in inflorescence); Calladium (flowers congested in inflorescence); Philodendron (flowers congested in inflorescence).

D—LONG-TONGUED INSECT FLOWERS

RANALES. *Ranunculaceae.*—Aconitum (spur); Actaea (no); Adonis (no); Aquilegia (spur); Atragene (no); Batrachium (no); Caltha (no); Clematis (no); Delphinium (spur); Eranthis (no); Helleborus (no); Isopyrum (no); Myosurus (no); Nigella (no); Pulsatilla (no); Ranunculus (no); Trollius (no). BERBERIDALES. *Berberidaceae.*—Berberis (no). RHOEDALES. *Papaveraceae.*—Chelidonium (no); Papaver (no). *Fumariaceae.*—Corydalis (spur); Dicentra (spur); Fumaria (spur). CRUCIALES. *Cruciferae.*—Alyssum (no); Arabis (no); Barbaraea (no); Berteroa (no); Brassica (no); Bunias (no); Cakile (no); Capsella (no); Cardamine (no); Cheiranthus (no); Crambe (no); Diplotaxis (no); Draba (no); Erophila (no); Erucastrum (no); Erysimum (no); Hesperis (no); Isatis (no); Lepidium (no); Lunaria (no); Matthiola (no); Nasturtium (no); Raphanus (no); Sinapis (no); Sisymbrium (no); Streptanthus (no); Teesdalea (no); Thelypodium (no); Thlaspi (no); Zilla (no). VIOLALES. *Violaceae.*—Viola (spur). *Resedaceae.*—Reseda (no). POLYGALALES. *Polygalaceae.*—Polygala (stam). SAXIFRAGALES. *Crassulaceae.*—Bryophyllum (no); Echeveria (no); Rhodiola (no); Sedum (no); Sempervivum (no). *Saxifragaceae.*—Chrysosplenium (no); Heuchera (p); Mitella (no); Parnassia (no); Saxifraga (p). CARYOPHYLLALES. *Caryophyllaceae.*—Agrostemma (no); Alsine (no); Arenaria (no); Cerastium (no); Coronaria (no); Dianthus (no); Gypsophila (no); Holosteum (no); Melandryum (no); Sagina (no); Saponaria (no); Scleranthus (no); Silene (no); Spergularia (no); Spergula (no); Stellaria (no); Tunica (no); Vaccaria (no); Viscaria (no). *Aizoaceae.*—Mollugo (no). *Portulacaceae.*—Calandrinia (no); Calyptridium (no); Claytonia (no). POLYGONALES. *Polygonaceae.*—Polygonum (no). CHENOPODIALES. *Phytolaccaceae.*—Phytolacca (no). GERANIALES. *Linaceae.*—Linum (no); Radiola (no). *Zygophyllaceae.*—Tribulus (no); Zygophyllum (no). *Geraniaceae.*—Erodium (no); Geranium (no). *Limnanthaceae.*—Limnanthes (no). *Oxalidaceae.*—Oxalis (no). LYTHRALES. *Lythraceae.—Lythrum* (no). *Onagraceae.*—Circaea (e); Epilobium (e); Gaura (e); Oenothera (e). THYMELAEALES. *Thymelaeaceae.*—Daphne (no); Dirca (no). BIXALES. *Cistaceae.*—Cistus (no); Helianthemum (no). CUCURBITALES. *Cucurbitaceae.*—Bryonia (e); Cucurbita (e). GUTTIFERALES. *Hypericaceae.*—Hypericum (no). TILIALES. *Tiliaceae.*—Tilia (no). MALVALES. *Malvaceae.*—Althaea (stam); Malva (stam); Sidalcea (stam); Sphaeralcea (stam). CUNONIALES. *Grossulariaceae.*—Ribes (e). *Hydrangeaceae.*—Deutzia (p); Philadelphus (p). ROSALES. *Rosaceae.*—Agrimonia (p); Amelanchier (p); Dryas (no); Fragaria (no); Geum (p); Potentilla (no); Prunus (p); Pyrus (e); Rubus (no); Spiraea (p). LEGUMINOSAE. *Papilionaceae.*—Amorpha (stam); Anthyllis (stam); Astragalus (stam); Colutea (stam); Coronilla (stam); Crotalaria (stam); Cytisus (stam); Galega (stam); Genista (stam); Hedysarum (stam);

TABLE 1.—*Continued*

Hippocrepis (stam); Indigofera (stam); Lathyrus (stam); Lens (stam); Lespedeza (stam); Lotus (stam); Lupinus (stam); Medicago (stam); Melilotus (stam); Onobrychis (stam); Ononis (stam); Ornithopus (stam); Oxytropis (stam); Phaca (stam); Phaseolus (stam); Pisum (stam); Sarothamnus (stam); Tephrosia (stam); Tetragonolobus (stam); Trifolium (stam); Ulex (stam); Vicia (stam); Vigna (stam). HAMAMELIDALES. *Hamamelidaceae.*—Hamamelis (p). SALICALES. *Salicaceae.*—Salix (no). CELASTRALES. *Celastraceae.*—Celastrus (p); Euonymus (p). RHAMNALES. *Rhamnaceae.*—Ceanothus (p); Paliurus (p); Rhamnus (p). *Vitaceae.*—Vitis (no). RUTALES. *Rutaceae.*—Dictamnus (stip); Ptelea (p); Ruta (p). SAPINDALES. *Sapindaceae.*—Aesculus (no). *Aceraceae.*—Acer (no). *Staphyleaceae.*—Staphylea (p). ERICALES. *Ericaceae.*—Andromeda (no); Arbutus (no); Arctostaphylos (no); Calluna (no); Epigaea (no); Erica (no); Gaultheria (no); Kalmia (no); Ledum (no); Monotropa (no); Phyllodoce (no); Pyrola (no); Rhododendron (no). *Vacciniaceae.*—Vaccinium (e). LOGANIALES. *Oleaceae.*—Jasminum (no); Syringa (no). APOCYNALES. *Apocynaceae.*—Apocynum (no); Nerium (no); Vinca (no). *Asclepiadaceae.*—Asclepias (stam); Stapelia (no). RUBIALES. *Rubiaceae.*—Asperula (e); Galium (e); Sherardia (e). *Caprifoliaceae.*—Linnaea (e); Symphoricarpus (e). ASTERALES. *Valerianaceae.*—Centranthus (e + spur); Valeriana (e); Valerianella (e). *Dipsacaceae.*—Dipsacus (e); Scabiosa (e). *Compositae.*—Achillea (e); Adenostyles (e); Agoseris (e); Antennaria (e); Anthemis (e); Arnica (e); Aster (e); Balsamorhiza (e); Bellis (e); Bidens (e); Calendula (e); Carduus (e); Carlina (e); Centaurea (e); Centromadia (e); Chaenactis (e); Chrysanthemum (e); Chrysocoma (e); Cichorium (e); Cirsium (e); Coreopsis (e); Crepis (e); Dahlia (e); Diplopappus (e); Doronicum (e); Echinops (e); Ericameria (e); Erigeron (e); Eriophyllum (e); Eupatorium (e); Gnaphalium (e); Grindelia (e); Gutierrezia (e); Haplopappus (e); Helianthus (e); Helichrysum (e); Hemizonia (e); Hieracium (e); Homogyne (e); Hypochoeris (e); Inula (e); Lactuca (e); Lampsana (e); Lappa (e); Layia (e); Leontodon (e); Lessingia (e); Madia (e); Malacothrix (e); Matricaria (e); Microseris (e); Monoptilon (e); Onopordon (e); Petasites (e); Picris (e); Pulicaria (e); Rudbeckia (e); Scorzonera (e); Senecio (e); Serratula (e); Silybum (e); Solidago (e); Sonchus (e); Stenotopsis (e); Tanacetum (e); Taraxacum (e); Tragopogon (e); Tussilago (e); Vernonia (e); Wyethia (e). GENTIANALES. *Gentianaceae.*—Erythraea (no); Gentiana (no); Limnanthemum (no); Menyanthes (no); Sweertia (no). PRIMULALES. *Primulaceae.*—Anagallis (no); Androsace (no); Cyclamen (no); Dodecatheon (no); Glaux (no); Hottonia (no); Lysimachia (no); Primula (no); Samolus (no); Soldanella (no); Steironema (no). *Plumbaginaceae.*—Armeria (no); Statice (no). CAMPANALES. *Campanulaceae.*—Adenophora (e); Campanula (e); Hedraeanthus (e); Jasione (e); Phyteuma (e); Specularia (e); Trachelium (e); Wahlenbergia (e). *Lobeliaceae.*—Downingia (e); Lobelia (e). POLEMONIALES. *Polemoniaceae.*—Collomia (no); Gilia (no); Langloisia (no); Leptodactylon (no); Linanthus (no); Navarretia (no); Phlox (no); Polemonium (no). *Hydrophyllaceae.*—Ellisia (no); Emmenanthe (no); Eriodictyon (no); Hesperochiron (no); Hydrophyllum (no); Miltitzia (no); Nama (no); Nemophila (no); Phacelia (no). BORAGINALES. *Boraginaceae.*—Amsinckia (no); Anchusa (no); Arnebia (no); Borago (no); Caccinia (no); Cynoglossum (no); Echinospermum (no); Echium (no); Eritrichium (no); Heliotropium (no); Lappula (no); Lithospermum (no); Lycopsis (no); Mertensia (no); Myosotis (no); Nonnea (no); Omphalodes (no); Onosma (no); Pulmonaria (no); Rindera (no); Symphytum (no). SOLANALES. *Solanaceae.*—Atropa (no); Datura (no); Hyoscyamus (no); Lycium (no); Mandragora (no); Nicandra (no); Nicotiana (no); Physalis (no); Solanum (no). *Convolvulaceae.*—Convolvulus (no). PERSONALES. *Scrophulariaceae.*—Antirrhinum (no); Bartschia (no); Chelone (no); Collinsia (no); Digitalis (no); Diplacus (no); Erinus (no); Euphrasia (no); Gerardia (no); Gratiola (no); Lathraea (no); Linaria (spur); Melampyrum (no); Mimulus (no); Pedicularis (no); Pentstemon (no); Rhinanthus (no); Scrophularia (no); Tozzia (no); Verbascum (no); Veronica (no). *Orobanchaceae.*—Orobanche (no). *Lentibulariaceae.*—Pinguicula (spur); Utricularia (spur). *Pedaliaceae.*—Martynia (no). *Acanthaceae.*—Acanthus (no); Rhinacanthus (no). LAMIALES. *Globulariaceae.*—Globularia (no). *Verbenaceae.*—Lippia (no); Verbena (no). *Labiatae.*—Agastache (no); Ajuga (no); Ballota (no); Blephilia (no); Brunella (no); Calamintha (no); Dracocephalum (no); Elsholtzia (no); Galeopsis (no); Glechoma (no); Hedeoma (no); Horminium (no); Hyptis (no); Hyssopus (no); Lamium (no); Lavandula (no); Leonurus (no); Lophanthus (no); Lycopus (no); Marrubium (no); Melissa (no); Melittis (no); Mentha (no); Micromeria (no); Moluccella (no); Monardella (no); Nepeta (no); Origa-

TABLE 1.—*Continued*

num (no); Phlomis (no); Physostegia (no); Pogogyne (no); Pycnanthemum (no); Rosmarinus (no); Salazaria (no); Salvia (no); Satureja (no); Scutellaria (no); Sphacele (no); Stachys (no); Teucrium (no); Thymus (no); Trichostema (no). BUTOMALES. *Butomaceae.*—Butomus (no). ALISMATALES. *Alismataceae.*—Alisma (no); Sagittaria (no). COMMELINALES. *Commelinaceae.*—Commelina (no); Tradescantia (no). LILIALES. *Liliaceae.*—Anthericum (no); Asparagus (no); Asphodelus (no); Bulbocodium (no); Calochortus (no); Camassia (no); Clintonia (no); Colchicum (no); Erythronium (no); Fritillaria (no); Gagea (no); Hemerocallis (no); Hyacinthus (no); Lilium (no); Lloydia (no); Majanthemum (no); Muscari (no); Narthecium (no); Paradisia (no); Polygonatum (no); Scilla (no); Smilacina (no); Streptopus (no); Tofieldia (no); Tulipa (no); Uvularia (no); Veratrum (no); Zygadenus (no). *Trilliaceae.*—Scoliopus (no). *Pontederiaceae.*—Eichornia (no). AMARYLLIDALES. *Amaryllidaceae.*—Allium (no); Brodiaea (no); Galanthus (e); Leucojum (e); Narcissus (e). IRIDALES. *Iridaceae.*—Crocus (e); Gladiolus (e); Iris (e); Sisyrinchium (e). AGAVALES. *Agavaceae.*—Yucca (no). ORCHIDALES. *Orchidaceae,*—Calypso (e); Cypripedium (e); Epipactis (e); Epipogon (e + spur); Goodyera (e); Gymnadenia (e + spur); Herminium (e + spur); Listera (e); Neottia (e); Nigritella (e + spur); Ophrys (e); Orchis (e + spur); Platanthera (e + spur); Spiranthes (e).

The mere fact that a bird has been observed to feed at a flower does not, of course, mean that it also pollinates that flower. Since it is not known in many cases whether the visits of the birds result in the pollination of the flowers, the list in Table 1 is necessarily uncritical. Some genera will have been included which are probably not pollinated by birds and hence are not in any true sense bird flowers. This may be the case with *Carica, Jacaratia, Helianthus, Tradescantia* and others. The defect which this factor of uncertainty introduces into the list of bird flowers in Table 1 is not serious, however, for the following reasons. Most flower-visiting birds, with the notable exception of the North American hummingbirds, are probably fairly specific, or at least not indiscriminate, in their relations to plants. Their bills are frequently highly specialized so as to suggest that they feed on a relatively limited number of flower forms. The available observational evidence points to the same conclusion (see Knuth and Loew, vol. 3, pt. 2, pp. 359 ff.). It is probable, therefore, that the majority of the genera listed in Table 1 part A contain species which are visited by birds with sufficient regularity to increase the selective advantage of ovule protection.

A total of 556 genera belonging to 102 families of flowering plants is listed in part A of Table 1. The condition of the inferior ovary is of widespread occurrence in 210 of these genera belonging to the Onagraceae, Cactaceae, Myrtaceae, Lecythidaceae, Melastomaceae, Combretaceae, Rhizophoraceae, Rosaceae, Loranthaceae, Vacciniaceae, Rubiaceae, Lobeliaceae, Gesneriaceae, Bromeliaceae, Musaceae, Zingiberaceae, Marantaceae, Amaryllidaceae, Iridaceae, Orchidaceae and many other families. The ovary is elevated on a stipe in 48 genera belonging to the Capparidaceae, Proteaceae, Passifloraceae, Caesalpiniaceae, Mimosaceae and several other families. A stamen column is developed as a sheath surrounding the ovary in 58 genera of Sterculiaceae, Bombacaceae, Malvaceae, Mimosaceae, Papilionaceae and other families. A spur is present in 17 genera distributed among such families as the Ranunculaceae, Vochysiaceae, Geraniaceae, Tropaeolaceae, Balsaminaceae, Marcgraviaceae and others. Certain other structural arrangements which might help to protect the ovules from the bill of the bird, as for example, the constriction of the corolla above the ovary or the armature of the ovary wall, may be recognized in 6 additional genera.

Special floral structures which apparently increase the protection of the ovules are present in 339 genera of bird-pollinated plants. That figure constitutes 61 per cent of the total number of genera listed as bird flowers in table 1. Another 217 genera, constituting 39 per cent of

the total number of genera in the list, have survived the visits of birds without the development of any apparent means of ovule protection other than the attachment of the ovules on the central axis of the ovary. Although the genera belonging to this class of unprotected bird flowers are scattered throughout a large number of families, by far the greatest concentration of them, namely 141, or 25 per cent of the total number of genera, belong to that great natural assemblage which includes the Ericaceae, Polemoniaceae, Bignoniaceae, Acanthaceae, Labiatae and their allies.

It should be of interest to compare the foregoing figures with similar statistics obtained from a large sample of insect-pollinated plants. For the purpose of making this comparison, a list of plant genera containing species which are visited and probably pollinated by insects with long probosces, particularly bees, wasps, long-tongued flies, moths and butterflies, was compiled from Knuth (1898–1899), Knuth and Loew (1904–1905) and unpublished records of the author. The list of long-tongued insect flowers was arranged according to the system of Hutchinson and annotated for the same floral structures that were considered in bird-pollinated plants. This list is presented as part D of table 1. The list given in part D overlaps to a small extent with the list in part A. This overlap is a consequence of the fact that certain genera include both bird-pollinated and insect-pollinated species and certain species may be both bird and insect-pollinated. Such duplication as exists between the list of bird flowers and the list of long-tongued insect flowers is not, however, a disadvantage in the statistical comparison of the two samples.

A total of 484 genera belonging to 79 families are recorded in table 1 part D. No special means of ovule protection can be recognized in 309 of these genera belonging to 59 families. The ovary is inferior in 125 genera, 70 of which belong to the Compositae. A stiped ovary is found in one genus of Rutaceae. A stamen column is present in 39 genera, of which 33 belong to the Papilionaceae. A spur exists in 10 genera distributed among the families Ranunculaceae, Fumariaceae, Violaceae, Scrophulariaceae, and Lentibulariaceae. Special morphological structures similar to those which were interpreted as means of ovule protection in bird flowers are present in a total of 175 genera of long-tongued insect flowers. This figure forms 36 per cent of the total number of genera in the class of long-tongued insect flowers.

The frequencies of the various means of ovule protection in bird flowers and in long-tongued insect flowers may be compared directly in table 2. This table shows that floral structures associated

TABLE 2. *The frequency of special ovule protection in bird and bat flowers as compared with long-tongued insect flowers*[1]

Pollination class	No. families	No. genera	Per cent genera						
			With ovule protection						Without protection
			Inferior ovary	Stiped ovary	Stamen column	Spur	Other	Total	
Bird flowers	102	556	37.8	8.6	10.4	3.1	1.1	61.0	39.0
Bat flowers	18	31	19.3	6.5	25.8	3.2	3.2	58.0	42.0
Long-tongued insect flowers	79	484	25.8	0.2	8.0	2.1	0.0	36.2	63.8

[1] A flower which possesses more than one type of ovule protection is counted only once in tables 2 and 3.

with ovule protection are nearly twice as frequent in bird flowers as in long-tongued insect flowers. It is also evident from the table that special means of ovule protection are present in a fairly large proportion of long-tongued insect flowers and absent in a fairly large proportion of bird flowers.

The significance of the comparisons given in table 2 may be increased by a study of the correlations existing within natural families and orders which include both bird-pollinated and insect-pollinated members. The inadequacy of our knowledge concerning the floral ecology of most systematic groups of plants constitutes a limiting factor in this entire discussion. Some suggestive correlations are, however, apparent with the information now available. An attempt will be made in the following paragraphs to show that variations in the relative positions of the ovules and nectar in some natural groups are correlated with differences in the mode of pollination.

The Capparidaceae, Moringaceae, Cruciferae and Resedaceae form a related group of families, according to Hutchinson (1926) and Brown (1938). In all of these families the nectar is secreted by glands on the receptacle at the base of the stamens (Brown, 1938). The Capparidaceae and Moringaceae, so far as we can judge at present, are largely pollinated by birds. The ovary is elevated on a stipe in these two families. The Cruciferae, which are pollinated by long-tongued insects, and the Resedaceae, which are regularly visited by beetles and long-tongued insects, lack a stipe or any other special type of ovule protection.

The Geraniales are a very natural order, according to Brown, in which the nectar glands are modified staminodia. Within this order the Balsaminaceae and Tropaeolaceae are pollinated by birds and possess a spur, whereas the Linaceae, Zygophyllaceae, Limnanthaceae and Oxalidaceae are pollinated by long-tongued insects and lack a spur.

The Nyctaginaceae are placed in the order Caryophyllales by Brown on the basis of the great similarities in the nectaries in both groups. The nectaries form a ring at the base of the stamens. Many of the Nyctaginaceae are probably bird flowers and possess a stamen column. The Caryophyllaceae, Portulacaceae, Polygonaceae and several other members of the order, on the other hand, are pollinated by long-tongued insects and lack a stamen column.

The Flacourtiaceae is a family which has retained generalized floral characters such as to suggest that it represents an ancestral type from which several extensive lines of evolution have been derived (Brown, *op. cit.*). Throughout the derivative orders, the Bixales, Tamaricales, Passiflorales, Cucurbitales, Violales, Capparidales, Cruciales and Salicales, the nectar is secreted from glands or disks on the receptacle (*loc. cit.*). The Flacourtiaceae are listed as insect flowers by Knuth and Loew. No special protection of the ovules exists in this family. In the Cochlospermaceae and Fouquieriaceae the nectary is a disk around the base of the ovary; these families are pollinated by birds and likewise lack special ovule protection. In the bird-pollinated Passifloraceae, however, the ovary is raised above the receptacular nectary by a stipe, while in the bird-pollinated Begoniaceae the nectar disk is above the ovary. Some tropical members of the Violaceae are probably pollinated by birds. Here we may recognize no ovule protection in some genera, stamen columns in others, and spurs in still others. The separation of the ovary from the nectary by a stipe in the bird-pollinated Capparidaceae has already been mentioned.

Within the Theales, the Marcgraviaceae, which are probably pollinated by birds, possess spurred flowers, whereas the Theaceae, which are probably not bird flowers, are not spurred.

In the Tiliales and Malvales the nectar is secreted at the base of the sepals (Brown, *op. cit.*). Many of the Tiliaceae are pollinated by insects and lack special

ovule protection. The related families Sterculiaceae, Bombacaceae and Malvaceae, on the other hand, are largely pollinated by birds and have a stamen column interposed between the ovary and the nectar.

The nectar gland is typically a disk around the base of the ovary in the Ericales (*loc. cit.*). This is the case in both the insect and the bird-pollinated species of the Ericaceae and in the bird-pollinated members of the Epacridaceae. In the Vacciniaceae, however, which are largely pollinated by birds (except the north temperate species), the nectary is seated above the ovary.

The large natural order Personales, in which the nectary generally surrounds the base of the ovary as in the Ericaceae (*loc. cit.*), includes numerous bird-pollinated types, particularly in the Bignoniaceae, Acanthaceae and Gesneriaceae. Bird-pollinated genera are known also in the Verbenaceae and Labiatae of the related order Lamiales. The absence of any means of ovule protection other than the attachment of the ovules to the central axis of the ovary has apparently not interfered with the success of the numerous bird-pollinated species belonging to these orders. An investigation of the flower pollination of these groups in relation to the problem of ovule protection would be most interesting. It may be significant that the ovary has become inferior once in this line, as it has also in the related but more generalized order Ericales. The bird-pollinated family Gesneriaceae includes some genera with superior ovaries and others with inferior ovaries. Among the genera with superior ovaries are some types with the corolla constricted just above the ovary.

Brown has suggested that the great rarity of inferior ovaries in the Ericales, Polemoniales, Personales, Lamiales and their relatives may be due to the attachment of the nectary to the base of the ovary where it would be buried if the ovary were to become inferior. It is of course axiomatic that a separation of ovary and nectary by the development of an inferior ovary, stiped ovary or stamen column could not take place, however strong the selective pressures for such a separation, unless the plants should mutate for a detachment of the nectary from the ovary. The nectary has in fact become disjoined from the base of the ovary and connected with the receptacle or corolla in certain genera belonging to at least three groups, the Ericales, Gesneriaceae and Verbenaceae, in this assemblage of orders, and in two of these groups inferior ovaries have developed without the submergence of the nectary (Brown, *op. cit.*).

In the natural assemblage of the Liliales, Alstroemeriales, Amaryllidales and Iridales the nectaries are glands in the cavities between adjacent carpels (*loc. cit.*). The Liliaceae, which are largely insect-pollinated, though including some bird-pollinated members, have superior ovaries, whereas the Alstroemeriaceae, Amaryllidaceae and Iridaceae, which are largely bird plants, have inferior ovaries with the nectar secreted above the ovary.

The correlations which have been described, though based on data which are not as adequate as might be desired, suggest that the hypothesis of special ovule protection in bird flowers is not without foundation. It will be desirable to review some further evidence, however, before attempting to derive any general conclusions.

Ovule Protection in Bat Flowers

The principal flower-visiting mammals are bats. The habit of visiting flowers is developed among the Fruit bats of the family Pteropodidae in the Old World tropics and among the Phyllostomatidae in the New World tropics. Bats belonging to these groups scratch the petals of *Musa, Ceiba, Bombax* and *Crescentia* with their claws (Pijl, 1936; Porsch, 1931). They likewise scratch the stamen column of *Adansonia* with their claws (Pijl, 1936). Bats tear up the flowers and eat the petals of *Bombax, Payena, Pallaquium*

and *Eugenia* (Porsch, 1937, 1941). To what extent the ovules of a flower would be damaged by the muzzle or teeth of bats in the absence of special protective structures is unfortunately not clear.

A list of known bat flowers was compiled from Pijl (1936) and Porsch (1935) and was annotated in the same way as the lists of bird and long-tongued insect flowers. This list of 31 genera in 18 families is presented as part B of table 1. The statistics for bat flowers are brought together with those for bird flowers and long-tongued insect flowers in table 2. It will be seen from these tables that bat flowers occur in many genera of plants which have also produced bird flowers, and that the total frequency of special ovule protection, allowing for sampling errors, is about the same in bat flowers and bird flowers.

The interpretation of these results cannot be undertaken without more detailed information concerning the relations of bats and flowers than is now available. It seems safe to conclude that special means of ovule protection are of widespread distribution in bat flowers. Whether the flower-visiting habits of bats have set up the selective pressures for the evolution of these features, however, or whether they have been inherited from a previous period of bird pollination, cannot be decided at the present time.

Ovule Protection in Beetle Flowers

The flower-visiting animals so far considered hover or clamber at the entrance to a flower and insert a bill, muzzle or extensile tongue into the floral chamber. The small size of beetles enables the whole animal to enter the flower. Here the beetle crawls about, chewing upon the various floral parts. Beetles are known to chew the carpels of *Rubus* (Heymons, 1915), the stigmas of *Magnolia* (Prantl, 1891), the ovaries of *Brassica* (Heymons, *op. cit.*) and the ovules and seeds of *Iris* (Knuth and Loew, 1904–1905). They eat the torus of *Rubus* (Heymons, *op. cit.*) and *Victoria* (Knuth and Loew, *op. cit.*). They likewise eat the anthers of *Ranunculus, Thalictrum, Cochlearia, Crambe, Silene, Rosa, Fragaria, Ulmaria, Spiraea, Crataegus, Pyrus, Philadelphus, Galium, Knautia, Inula, Crepis, Phacelia, Convolvulus, Mentha, Primula, Polygonum* and *Trillium* (Knuth; Knuth and Loew). Beetles chew on the petals of *Pyrus, Spiraea, Sambucus* and *Rosa,* and gnaw the inner floral parts of many other plants (*loc. cit.*).

The ovules can be protected from the chewing mandibles of the beetles if the surface upon which the beetles crawl within the flower is raised above the ovules. This condition is fulfilled by several different arrangements. The floral chamber may be raised above the ovules by the development of a torus surrounding the carpels (the condition known as perigyny, fig. 2, a–e), by the development of an inferior ovary (f–g), or by the close congestion of the flowers in a head or spike (h).

In order to study the occurrence of

Table 3. *The frequency of special ovule protection in beetle flowers as compared with long-tongued insect flowers*

Pollination class	No. families	No. genera	Per cent genera				
			With ovule protection				Without protection
			Inferior ovary	Perigyny	Congestion of flowers	Total	
Beetle flowers	17	35	45.6	25.8	8.6	80.0	20.0
Long-tongued insect flowers	79	484	24.8	3.7	0.0	28.5	71.5

these features a list of known or presumed beetle flowers was compiled from Delpino (1875), Knuth, (1898–1899), Knuth and Loew (1904–1905), Schrottky (1910), Diels (1916) and Grant (1950a, unpubl.). The plant genera, as annotated for the existence of perigyny, inferior ovaries or close congestion of the flowers in an inflorescence, are listed in part C of table 1. The data obtained for the list of beetle flowers were next compared with similar data on long-tongued insect flowers in table 3. The great variety of conditions subsumed by the term perigyny made it necessary to consider each case on its individual merits in compiling tables 2 and 3. A flower which is perigynous in relation to a crawling bee-

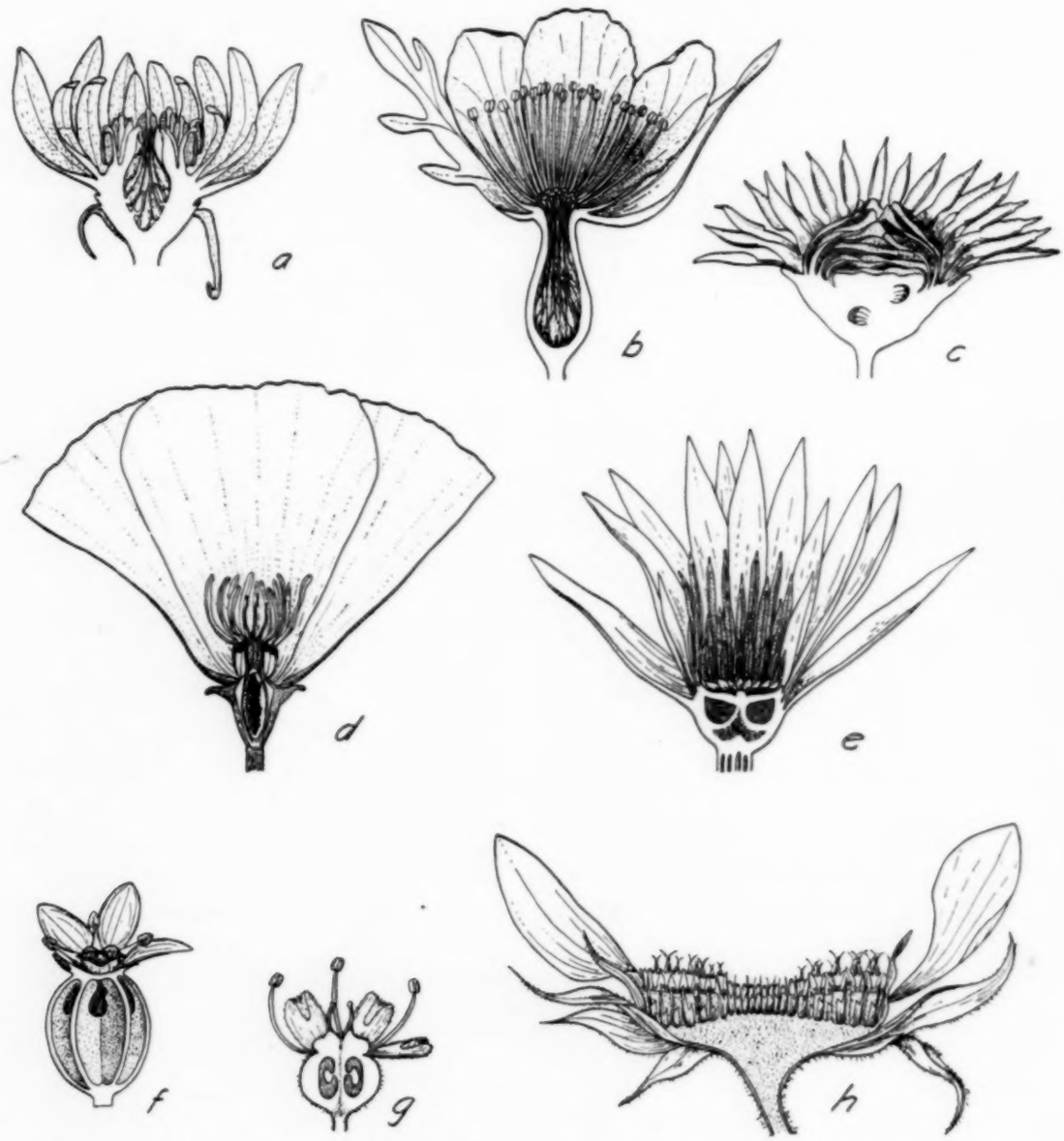

FIG. 2. Longisectional views of beetle flowers showing various means of ovule protection: perigyny (a–e); inferior ovary (f–g); close grouping of flowers in a head (h).

a—*Calycanthus occidentalis* (Calycanthaceae); b—*Rosa canina* (Rosaceae); c—*Eupomatia laurina* (Eupomatiaceae); d—*Eschscholtzia californica* (Papaveraceae); e—*Nymphaea coerulea* (Nymphaeaceae); f—*Sambucus australis* (Caprifoliaceae); g—*Dorema ammoniacum* (Umbelliferae); h—*Helianthus annuus* (Compositae).

Illustrations redrawn from Engler and Prantl, except (a) and (d) which are original.

tle, for example, may or may not have its ovules protected from a probing bird. As a result of these and similar considerations, a few differences have arisen between the treatment of long-tongued insect flowers in tables 2 and 3.

The 35 genera of beetle flowers, as shown in table 3, have a significantly higher proportion of cases of ovule protection than the genera of long-tongued insect flowers. Eighty per cent of the genera of beetle plants have the ovules concealed beneath the floor of the floral chamber, whereas only 28 per cent of the genera of long-tongued insect flowers possess the same devices. It is of interest to note that the difference in floral structure between the beetle-pollinated Nymphaeaceae and the non-beetle-pollinated Ranunculaceae, to which they are related (Hutchinson, 1924; Brown, 1938), is largely the difference between the presence and absence of perigyny. The suggestion of a similar correlation between perigyny and beetle pollination exists also within the tribes Rosoideae and Spiraeoideae of the family Rosaceae.

The existence of ovule protection in beetle flowers acquires considerable phylogenetic significance in view of the hypothesis that the original flowering plants and their immediate ancestors were pollinated by beetles (Diels, 1916; Grant, 1950a). The reproductive organs of a Jurassic cycad, *Cycadeoidea* (Bennettitales), were of a type which suggests beetle pollination (Grant, 1950a). The ovules were protected in this plant by the development of a series of overarching scales. Dr. G. L. Stebbins has suggested to the author that the progressive fusion and closure of the ovule-bearing organs in certain seed ferns between Triassic and Jurassic time, viz., *Pilophorosperma*, *Gristhorpia* and *Caytonia* (Thomas, 1936), may have taken place in response to the visits of beetles. In still another evolutionary line of the Mesozoic era, the line namely that led to the flowering plants, the development of a closed carpel may have represented a successful method of ovule protection in relation to beetle pollination.

Ovule Protection in Long-Tongued Insect Flowers

It has long been known (Darwin, 1876; Knuth, 1898–1899; Knuth and Loew, 1904–1905) that certain bees habitually bite holes in corollas for the illegitimate extraction of nectar. The chief offenders are *Bombus* and *Xylocopa*, though *Apis*, *Trigona* and probably other bees may also occasionally mutilate corollas. The habit is known also in *Vespa*, *Odynerus* and other wasps (Vespoidea), as well as in ants. A list of recorded cases of the perforation of the corolla and theft of the nectar by bees and wasps, which was compiled by the author, included about 50 genera of angiosperms, all of which store nectar in a deep tube. The conclusion reached by the classical floral biologists that the habit of breaking into flowers through the side of the corolla is correlated with the possession of a proboscis too short for the extraction of nectar in the normal fashion seems well justified. This conclusion is strengthened by the fact that Hummingbirds, Sunbirds, Honeyeaters, Flower-peckers and other kinds of birds exhibit the same habit in relation to tubular flowers in nearly all parts of the world (Knuth and Loew, 1904–1905; Porsch, 1924, 1927b, 1929; Tiwary, 1926; Malme 1923; Swynnerton, 1915a, 1915b; Lowe, 1896; Moore, 1878).

No case is known to the author, however, of damage to the inner organs of a flower by any adult bee, wasp, fly, moth or butterfly. As shown in tables 2 and 3, most flowers which are regularly visited and pollinated by these insects have superior ovaries devoid of any special means of ovule protection. The existence of a small contingent of long-tongued insect flowers with protected ovules is, nevertheless, definitely indicated by these same tables. A more detailed examination of the exceptional cases, with the aid of Engler and Prantl (1888–1898) and other sources, may be instructive.

The Papilionaceae have been cited since the time of Hermann Müller as the bee flowers par excellence. There can be no doubt that most temperate genera of this family are indeed pollinated by bees; the most generalized members of the order Leguminosae, however, are woody plants of the tropics which are pollinated by birds. In the woody tropical Papilionaceae a stipe, or more usually a stamen column, is associated with bird pollination. It seems not unlikely that the stamen column of the herbaceous temperate Papilionaceae represents a character which has been inherited from bird-pollinated ancestors.

Polygala is another bee plant with a stamen column. The most generalized members of the Polygalaceae are again woody and tropical. Bird pollination occurs in the related family Vochysiaceae and probably in the tropical Polygalaceae as well. It may well be that the stamen column was developed in relation to bird pollination in the tropical members of the family and was retained in *Polygala* during the extension of that genus into northern latitudes and the subsequent change-over to insect pollination.

The Malvaceae also have a stamen column, although bees are the important pollinators of several north temperate genera. Most of the genera of this family, however, as well as the closely related families Sterculiaceae and Bombacaceae, are tropical bird plants. The stamen column of the temperate Malvaceae is probably best explained, therefore, as a vestige of a previous period of bird pollination.

Vaccinium has an inferior ovary, although in the north temperate zone it is pollinated by bees. The family Vacciniaceae as a whole, however, is largely concentrated in tropical areas and pollinated by birds. These considerations suggest that the north temperate species of *Vaccinium* have been derived from bird-pollinated ancestors. If this hypothesis is correct, then the inferior ovary of the bee-pollinated species of *Vaccinium* is correlated, not with their present mode of pollination, but with a former period of bird pollination.

The Orchidaceae of Europe and North America, which are largely pollinated by bees, wasps, lepidopterans and flies, possess an inferior ovary. The closest relatives of the Orchidaceae, the Haemodorales (Hutchinson, 1934) and Zingiberales (Rendle, 1904; Wettstein, 1924), are, however, characteristic bird flowers of tropical latitudes. If, as seems probable, the north temperate orchids are derived from tropical bird-pollinated ancestors, the inferior ovary of the northern species may represent a retention of a character acquired under conditions of pollination very different from those which now prevail.

The north temperate species of Iridaceae are pollinated by bees and lepidopterans and have an inferior ovary. The family has its main center of distribution in south temperate and tropical areas, however, where its members are predominantly pollinated by birds. It seems probable that an inferior ovary was developed in this family under conditions of bird pollination and was retained in the species which colonized northern areas and became pollinated by long-tongued insects.

No ecological classification of flower types that has ever been proposed has satisfactorily dealt with the huge and unique family Compositae. The useful classification of flower types according to their principal agent of pollination, which was initiated by Delpino (1875) and further developed by Müller (1881), simply made an exception of the Compositae by either ignoring them or by calling them social flowers and placing them in a special class of their own. It is evident that the latter course, in which Müller was followed by Knuth (1898), merely evades the fundamental problem of what causal factors have moulded the reproductive organs of the Compositae. Flowers aggregated into dense inflorescences, viz. social flowers, may be found in all pollination classes: wind flowers (*Betula*); water

flowers (*Phyllospadix*); snail flowers (*Alocasia*); beetle flowers (*Spiraea*); fly flowers (*Arum*); wasp flowers (*Ficus*); bee flowers (*Gilia*); butterfly flowers (*Centranthus*); bird flowers (*Protea*); bat flowers (*Freycinetia*); and marsupial flowers (*Dryandra*). The statement that the Compositae are "social flowers" is remarkably uninformative.

In table 1 some 70 genera of Compositae were included in the class of long-tongued insect flowers, and there can be no doubt that many if not most of the animal-pollinated Compositae of temperate latitudes are in fact pollinated by bees and other long-tongued insects. The prevailing yellow and blue colors, sweet-scented nectar, and in many cases deep tubular corollas are characters which elsewhere among flowering plants are associated with pollination by bees (Grant, 1950b). It is equally, true, however, that the aggregation of the small flowers into dense heads and the inferior position of the ovaries do not suggest contrivances for pollination by bees or other long-tongued insects.

The elaboration of a key idea of Delpino (1875) may provide a clue as to the ecological classification of the composite flower. Delpino recognized two types of beetle flowers, which he designated respectively as the *Magnolia* type and the *Hydrangea* type. The *Magnolia* type consists of large solitary flowers visited by beetles. According to Delpino, this type is exemplified by such genera as *Magnolia, Nelumbium, Nymphaea, Victoria, Euryale, Paeonia, Calycanthus* and *Eupomatia*. In the *Hydrangea* type, by contrast, a dense inflorescence composed of many small flowers is supposed to be visited and pollinated by beetles. Delpino cited as examples of this group *Hydrangea, Cornus, Ligustrum, Fraxinus, Viburnum, Sambucus, Crataegus* and *Ornithogalum*.

Subsequent research is tending to confirm Delpino's postulation of a beetle-pollinated *Magnolia* type of flower (Grant, 1950a). It is also well established that inflorescences of the *Hydrangea* type are abundantly visited by beetles (Knuth; Knuth and Loew). It seems probable from investigations now in progress that such inflorescences are in some cases also pollinated by beetles. The close congestion of the flowers enables the beetles to effect pollination in the process of crawling over the surface of the inflorescence and feeding upon the nectar and tissues of the individual flowers. The ovules are protected from the ravages of the beetles by the close grouping of the flowers and by the inferior position of the ovaries.

The occurrence of these very features in the inflorescence of the Compositae is highly suggestive. Beetles are frequently present on the heads, where they crawl over the disk and feed on the nectar and floral tissues; and in species with shallow corolla tubes and low stigmas they may even pollinate the flowers (Knuth; Knuth and Loew; Grant, unpubl.). These facts suggest that the Compositae may belong in Delpino's class of beetle-pollinated inflorescences.

The traditional practice of placing the Compositae at the peak of the system of classification of angiosperms has tended to obscure the fact that this family is very ancient. Some patterns of geographical distribution are known in the Compositae which can scarcely be explained on the basis of any age less than Cretaceous (Stebbins, 1939). Furthermore, a fossil inflorescence is known from Upper Cretaceous sediments of eastern North America which was identified by both Gray and Stebbins (*loc. cit.*) as a member of the Compositae-Helianthieae. Now beetles were the only important flower-visiting insects in existence during the Cretaceous period (see Grant, 1950a). The sparse available facts suggest that the Compositae may have acquired their fundamental floral characters under conditions of beetle pollination during the Mesozoic era. Their present features of color, odor and shape of corolla may have been superimposed on the basic characters during a change-over to bird pollination in the

tribe Mutisieae and to bee pollination in the Cynareae and other tribes after the beginning of the Tertiary period.

The arguments set forth in this section seem to justify the conclusion that many cases of ovule protection in long-tongued insect flowers are vestigial in the sense that they pertain not to contemporary conditions but to a former period of bird or beetle pollination. The deduction of the cases which were specifically mentioned, the Papilionaceae, Polygalaceae, Malvaceae, Vacciniaceae, Orchidaceae, Iridaceae and Compositae, from the list of long-tongued insect flowers in part D of table 1 would of course result in an even greater contrast in the frequencies of ovule protection in bird or beetle flowers as compared with long-tongued insect flowers than was indicated by tables 2 and 3. A recalculation of the data after deduction of the aforementioned families would lead to the estimate that only 9 or 10 per cent of long-tongued insect flowers have inferior ovaries, that only 0.3 per cent have stamen columns, and that 86 or 87 per cent have no special means of ovule protection.

Adaptation

In the preceding pages several correlations have been noted between the mode of pollination of flowers and certain modifications in their form. The purpose of the final section of this paper is to inquire whether those modifications in the form of flowers are in fact adaptations in relation to the process of pollination.

The condition of the inferior ovary is very widespread in the angiosperms. A survey of Hutchinson (1926–1934) reveals that this condition is present in 82 of the 332 families of angiosperms recognized by that author. The transition from a superior to an inferior ovary within a natural family or order, where it has been studied, suggests that the process is a gradual and sustained one, and hence probably guided by natural selection (see Eames, 1931, *re* Ericales; MacDaniels, 1937, *re* Rosaceae; Stebbins, 1940, *re* Compositae; Mattfeld, 1921, *re* Caryophyllaceae). A satisfactory hypothesis concerning the adaptive value or values of the inferior ovary has not, however, been offered.

The view tentatively adopted in the present paper is that the inferior ovary is, in many instances at least, an adaptation for ovule protection in flowers pollinated by birds or beetles. The evidence for this view is largely indirect and consists of a knowledge of the destructive pollinating habits of birds and beetles combined with a correlation between the presence of an inferior ovary and pollination by those animals. Further evidence bearing upon this question is obtained from a study of the mode of pollination in the 82 angiospermous families in which the ovary has become inferior.

The results of this survey are given in table 4. No data on the method of pollination are available for 28 little known families of tropical or south temperate latitudes (i.e., Grubbiaceae, Lowiaceae, Hypoxidaceae, etc.). This leaves 54 families with inferior ovaries in which the mode of pollination is known. Bird pollination is certainly or very probably present in 40 of these families and beetle pollination in 10 others. In only 4 of the 54 families with partly or wholly inferior ovaries can pollination by long-tongued insects be considered the basic condition (Elaeagnaceae, Portulacaceae, Saxifragaceae, Valerianaceae). Pollination by birds or beetles is present, therefore, in about 93 per cent of the angiospermous families with inferior ovaries.

Table 4. *Mode of pollination in the 82 families of flowering plants with inferior ovaries*

Agent of pollination		No. of families	Total	
Unknown		28	28	
Birds	certain	35		
	probable	5	40	50
Beetles	certain	8		
	probable	2	10	
Long-tongued insects		4	4	

The question may be raised whether the inferior ovary can have any other adaptive value than the protection of the ovules in flowers visited by destructive animals. It is possible that in some cases the inferior ovary may be related to fruit dispersal rather than to ovule protection. The elaborate dispersal mechanisms of the Compositae, for example, consist of modifications of the calyx and other parts fused to the top of the inferior ovary. The pomaceous fruits of the Rosaceae with inferior ovaries also secure the dispersal of the seeds in the intestinal tracts of frugivorous animals (see Ridley, 1930). Within the Bromeliaceae and Ericales, inferior ovaries are generally correlated with berry-like fruits and superior ovaries with capsular fruits.

The correlation of inferior ovaries with the dispersal mechanisms of the fruit is, however, apparently restricted to a few special cases. Edible berries are produced by both the hypogynous and epigynous Liliales. Berries are common among groups with superior ovaries and capsular fruits are frequent in plants with inferior ovaries. The existence of leafy calyces of a generalized type in both the Compositae and Rubiaceae suggests that the modifications of this floral part as a dispersal organ occurred after the inferior ovary had already been established in these families. The evidence does not suggest that inferior ovaries evolved in response to the requirements of the fruits in these two families at least.

The foregoing remarks do not, of course, rule out the possibility of a correlation between the inferior ovary and the ecology of the fruit. In some groups the inferior ovary may be related to the character of the fruit. In other groups a selection for rapid development of the flower may have resulted in the general fusion of floral parts, including the fusion of the ovary with surrounding tissues (Stebbins, 1950, ch. xiii). In the great majority of cases, however, the inferior ovary appears, on the evidence now available, to be an adaptation for ovule protection in bird and beetle-pollinated flowers.

The frequency of spurred flowers is not significantly greater in plant genera pollinated by birds than in genera pollinated by long-tongued insects (cf. table 2). Spurs are associated with pollination by Lepidoptera and Hymenoptera in *Aconitum*, Fumariaceae, *Centranthus. Linaria, Utricularia* and some Orchidaceae, about as much as with pollination by birds in Vochysiaceae, *Tropaeolum, Impatiens, Cuphea*, Marcgraviaceae, *Couepia, Tamarindus, Melianthus* and some Orchidaceae. These facts occasion some doubt as to whether the spur serves as a device for the protection of the ovules in bird flowers or whether it is simply a deep nectar-storing vessel from which animals with elongated mouths, both birds and insects, can feed. A detailed consideration of the function of the spur in *Aquilegia* will emphasize the difficulties of the hypothesis that the spur is an adaptation for ovule protection.

The blue-flowered species of *Aquilegia* in Europe, *A. vulgaris* and *A. alpina*, are pollinated by bumble-bees (Knuth); the red-flowered North American species, *A. canadensis* and *A. formosa*, are pollinated by hummingbirds (Knuth and Loew; Grant, unpubl.); and the yellow-flowered Mexican species, *A. longissima*, is probably pollinated by hawk-moths (Knuth and Loew). The proportions of the spur correspond with the nature of the sucking organ in the pollinating animal. The spur in the columbines pollinated by bumble-bees is about 2 cm. long with a broad entrance; in the forms pollinated by hummingbirds it is about 2 cm. long with a relatively narrow entrance; and in the flowers visited by hawk-moths the spur is about 12 cm. long and very slender (Munz, 1946).

The spur of *Aquilegia* is probably derived from a spurless honey leaf such as is found in *Aquilegia ecalcarata, Isopyrum* and other related members of the Ranunculaceae (Anderson and Schafer, 1931; Munz, *op. cit.*). The difference between

spurred and spurless flowers in *Aquilegia vulgaris* is due to the action of a single gene (J. Clausen, unpubl.). Since the spurless flowers of *Isopyrum, Anemone* and related members of the Ranunculaceae are very generally visited by bees, flies and beetles, but not by birds, it is improbable that the genes for a spur should have been established in the ancestors of *Aquilegia* due to selection in relation to bird visits. The spur in *Aquilegia,* therefore, even though it may guide the bill of the bird away from the carpels and hence save the ovules from some damage, cannot be regarded as primarily an adaptation for ovule protection.

The correlation between the presence of stiped ovaries or stamen columns and pollination by birds is very high (cf. table 2). The correlation between perigynous flowers with the ovules buried beneath a floor-like surface and pollination by beetles is likewise very high (table 3). These structures, by separating the mouthparts of the animals from the ovules, do in fact protect the ovules from damage caused by the pollinating animals, and it seems probable that they have been selected for the protective function which they perform.

The probability of this conclusion, as applied to the stamen column, is increased by the observation that this structure apparently never occurs in a flower with an inferior or a stiped ovary (cf. table 1). The stamen column and the stiped ovary are mutually exclusive within some genera of the Mimosaceae, within that family as a whole, and within the Papilionaceae. There is no obvious explanation of these facts on developmental grounds. Fused stamens occur together with inferior ovaries in the Compositae and Lobeliaceae which proves that a developmental correlation is possible; but here the fusion involves only the anthers and does not extend to the base of the stamens where it would protect the ovary.

The negative correlation between stamen columns and stiped or inferior ovaries, while inexplicable on an ontogenetic basis, is in complete agreement with the hypothesis of a selective origin of these structures. The selective pressure for ovule protection in an unprotected bird flower would be relieved in each species by the development of any one of several possible protective devices. The establishment of a single effective means of ovule protection in any species of bird-pollinated plant would, by relieving the selective pressure, abolish the mechanism for the accumulation of mutations leading to any other protective structure. A multiplication of adaptations for the protection of the ovules in bird flowers seems to be excluded by the theory of selection. The absence of bird flowers with both stamen columns and stiped or inferior ovaries therefore strengthens the conclusion that those structures are adaptations for ovule protection.

An experimental verification of the hypothesis of the adaptive value of ovule protection in bird and beetle flowers is greatly to be desired. Such an experimental attack might be possible in the Rosaceae. The variation in the degree of inferiority of the ovary in the tribe Pomoideae of that family, together with the fact that such morphologically dissimilar types as *Pyrus* sect. *Sorbus* and *Amelanchier* have fairly good chromosome homology and can hybridize to produce some viable F_2 progeny (Sax and Sax, 1947), indicates that the evolution of the position of the ovary in this tribe has proceeded at a more rapid rate than the evolution of genetic barriers. The experimental methods of genetics might well be combined with the observational methods of floral ecology in a study of the evolution of ovule protection in the Pomoideae. The Melastomaceae is another group which is apparently undergoing active evolution with respect to the position of the ovules. The ovary is superior in some genera, borne on a stipe in one genus, partly fused to the floral tube in some other genera, and inferior in varying degrees in the rest of the family. Even within a single genus, such as *Mouriria,* various

types and stages of inferior ovary may be recognized on anatomical grounds (Morley, unpubl.). Experimental studies on the adaptive value of an inferior ovary might be carried out in this family. Some species of *Simaba* (Simarubaceae) in South America have stiped ovaries and other species in the same area have sessile ovaries. An experimental investigation of the stiped and unstiped ovaries when exposed to bird visits under uniform conditions might be very interesting.

A satisfactory experiment to investigate the adaptive value of ovule protection might include the following elements. Let two related plant species differing in the position of the ovules be crossed. The second hybrid generation, segregating for ovule protection, could next be exposed to bird or beetle visits under uniform external conditions. The number of sound seeds harvested on the various phenotypes might then be used as an indication of the relative survival value of the genes governing the different positions of the ovules. The demonstration that plants with ovules borne in a protected position set a significantly different number of sound seeds than plants not so protected, with due allowances for the variable factors involved, would constitute strong evidence concerning the relative adaptive values of the respective genotypes.

The purpose of the present paper is to pose a general problem and state some preliminary conclusions as a basis for particular and experimental studies. The principal conclusion to which we are brought is that the phenomenon of ovule protection is of widespread occurrence among flowering plants, like mimicry in the insect world. That phenomenon can provide the biologist with materials for experimental studies on the origins of adaptations by means of natural selection.

Summary

Flower-visiting birds commonly injure the inner floral parts of plants in probing for nectar. The separation of the nectary from the ovary accomplishes the protection of the ovules from the bill of the bird. The separation of nectary and ovary is attained in several different ways. The ovary may be buried beneath the nectary as an inferior ovary, or elevated above it on a stipe, or a stamen column may be developed as a sheath between the ovary and the nectary. One or the other of these devices is found in the majority (58 per cent) of known bird flowers. The numerous bird-pollinated members of the Bignoniaceae, Acanthaceae, Verbenaceae and their allies form an exceptional group of bird flowers, constituting 25 per cent of the total number of bird flowers, in which the ovary and nectary are not separated and the ovules are apparently not protected by any special arrangement other than attachment on the central axis of the ovary.

Flower-visiting beetles commonly chew up the inner parts of flowers. The protection of the ovules from the chewing mandibles of the beetles is accomplished by the elevation of the floral chamber above the ovules. Several modifications of the form of the flower may prevent the beetles from crawling into the region of the ovules. The floral chamber may be elevated above the ovary by the development of a perigynous torus or an inferior ovary, or the flowers may be closely aggregated in an inflorescence so as to form a surface above the ovules. The majority (80 per cent) of known beetle flowers possess one or the other of the foregoing arrangements.

Adult bees, wasps, flies, moths and butterflies are not known to injure the inner parts of flowers in sucking nectar. Most flowers pollinated by long-tongued insects (64–72 per cent) lack the special structural modifications characteristic of bird and beetle flowers or, if they possess them, have apparently retained them as vestiges of a previous period of bird or beetle pollination (15–23 per cent).

In the light of the foregoing statements, the fact that 93 per cent of the families of angiosperms with inferior ovaries have

bird or beetle pollination suggests that the inferior ovary may, in many cases at least, be an adaptation for ovule protection under conditions of bird or beetle pollination. The high correlation between the presence of either stiped ovaries or stamen columns and bird pollination indicates that those structures may also be adaptations for ovule protection in bird flowers. The close association of perigynous flowers, a relatively infrequent condition in angiosperms, with beetle pollination suggests that perigyny may represent an adaptation for ovule protection in beetle flowers. The closed carpel may itself represent the original form of ovule protection in relation to beetle pollination in flowering plants.

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DEVELOPMENTAL RESTRICTIONS ON RECOMBINATION IN *NICOTIANA*[1]

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INTRODUCTION

Recombinations of certain characteristics of one parent with those of another are not found in the F_2 generation of some interspecific crosses. Among possible contributory causes to this restriction the following have been discussed (Anderson, 1939, 1949; Dempster, 1949): (1). Disharmonies—structural, genetic or physiological—which lead to differential elimination of genetic recombinations in gametes or zygotes formed by the F_1; (2). Pleiotropy; (3). Linkage; (4). Multifactorial control of the hereditary differences.

Analysis of segregation for corolla shape differences in populations derived from the hybrid *Nicotiana Langsdorffii* × *N. alata* has been used as illustrative material in Anderson's studies. Supplementary to the writer's investigations on the inheritance of quantitative characters in the cross *N. Langsdorffii* × *N. Sanderae* (Smith, 1937, 1943), developmental studies were made on the corollas of parental species, F_1 and representative F_2's. The ontogenetic relationships observed have a direct bearing on the problem of recombination restrictions and provided the basis for conclusions reached in the present paper.

MATERIALS AND METHODS

Inbred strains of *Nicotiana Langsdorffii* Weinm. (n = 9) and *N. Sanderae* Sander var. Sutton's Scarlet (n = 9) were used as parental species in these experiments (fig. 1). *N. alata* was one of the parents of *Sanderae* and the corolla of variety Sutton's Scarlet approaches *alata* in size. The characteristics studied were length of the corolla tube and length of the corolla limb as measured for the longest lobe. These features constitute two of the more conspicuous interspecific quantitative differences that can be readily investigated genetically. The F_1 *Langsdorffii* × *Sanderae* is intermediate in corolla size and shape, is partially pollen fertile, and sets abundant seed from either self-pollination or crossing reciprocally with the parental species.

Developmental studies were made by collecting flowers in many stages of growth, from small buds to maturity (fig. 2), and measuring them immediately. Approximately 150 corollas were measured for each of the parents and F_1; and, on an average, 50 for each of 20 F_2 segregants. All measurements were made with a millimeter rule, those above 15 mm. without magnification and those below at 7 ×. The lower limit of bud size was taken arbitrarily as that in which the tube was 5.5 mm. long. Consistent data from smaller buds were difficult to obtain with this technique, but reliable measurements on earlier stages could no doubt be made with more refined methods.

The data obtained were plotted on a double logarithmic grid with tube length on the ordinate and limb length on the abscissa. In figures 3 through 7 log. tube and log. limb signify a log. plot of the measurements in millimeters. Straight line relationships resulted that could be fitted satisfactorily by eye.

An area of possible recombination for the F_2 was projected on the basis of recombination of differentiating characteristics of the parental relative growth curves. Recombination areas for each of

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the backcrosses were constructed in a similar manner using the relative growth curves of the F_1 and recurrent parent. The distribution of measurements of mature corollas of F_2 and backcross segregants was plotted and related to the area of recombination postulated on the basis of developmental studies.

Results

Relative growth curves of the parental species and F_1 are shown in figure 3. The curve of the F_1 is labeled F″F′F, of *Langsdorffii* L″L′L and of *Sanderae* S″S′S. Clearly, throughout the major period of corolla development, the relative increase in tube length to limb length, i.e., the slope, is the same in all three curves. The tube elongates about two and a half times faster than the limb from early bud stage until just before anthesis when a sharp "turning point" (L′, F′ and S′) is reached. At this point there is almost complete cessation of tube elongation and a simultaneous rapid increase in the length of the limb until maturity is reached (see Nagel, 1939, for growth rates of *Langsdorffii* and *alata*).

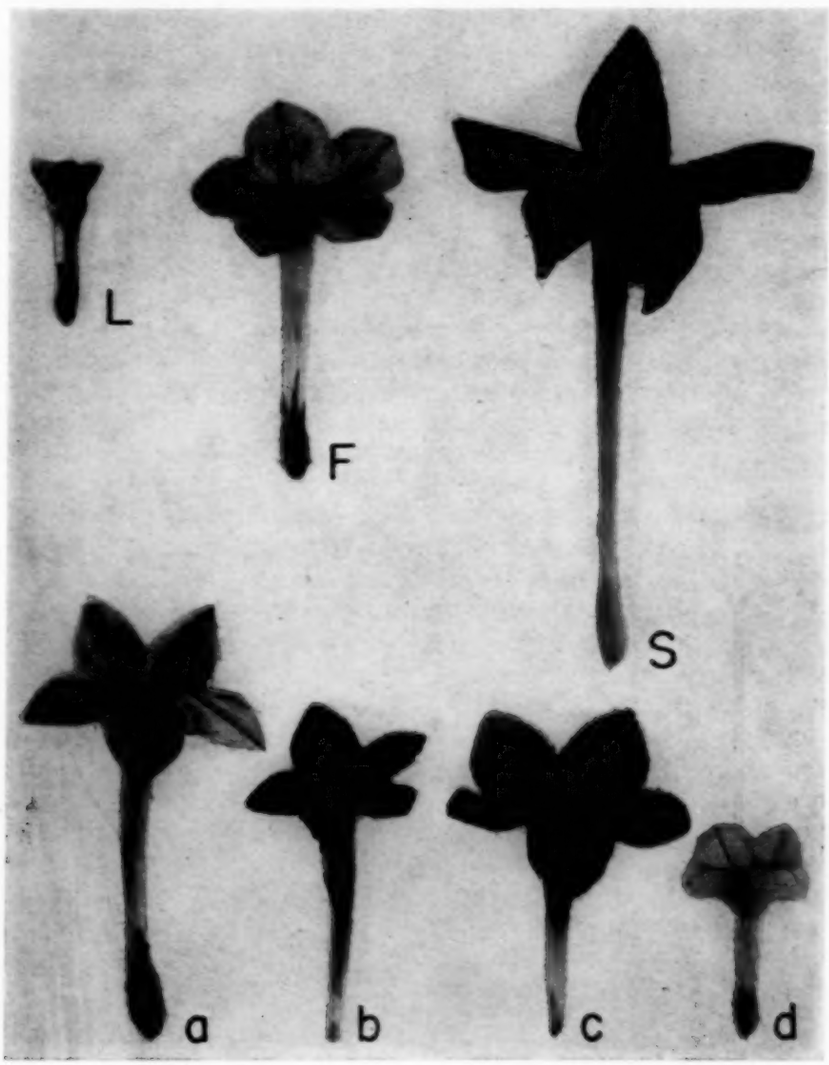

Fig. 1. Corollas ×-⅞ of: L—*Nicotiana Langsdorffii*, F—F_1 *Langsdorffii* × *Sanderae*, S—*N. Sanderae*, a—large F_2, b—F_2 with relatively long tube and short limb (39–3), c—F_2 with relatively short tube and long limb (39–36), d—small F_2. Relative growth curves of b and c are shown in figure 4.

The chief dissimilarities between the curves of *Langsdorffii* and *Sanderae* are: (1). A different proportion of tube to limb is established early in development, at a younger stage than studied here. When the tube is 5.5 mm. long the limb of *Langsdorffii* is 2.05 mm. (L″) while that of *Sanderae* (S″) is 5.40 mm. (table 1). (2). The amount of elongation that takes place before the turning point is different. In *Langsdorffii* this critical point is reached when the tube is about 19 mm. long (L′) and in *Sanderae* when it is 62 mm. (S′). After the turning

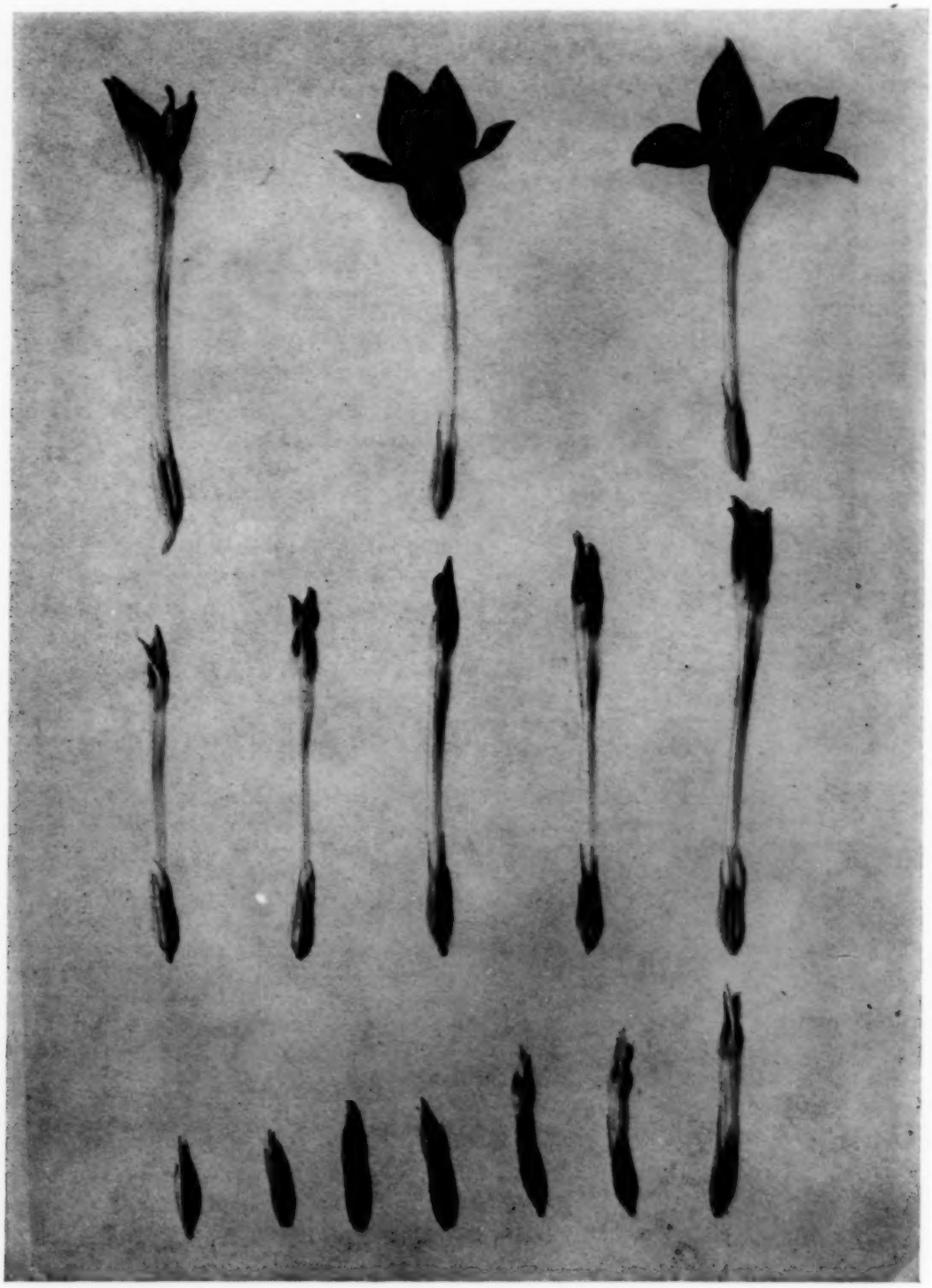

FIG. 2. Stages in corolla development in *N. Sanderae*, ×-⅗. The "turning point" stage is shown at the extreme right of the middle row.

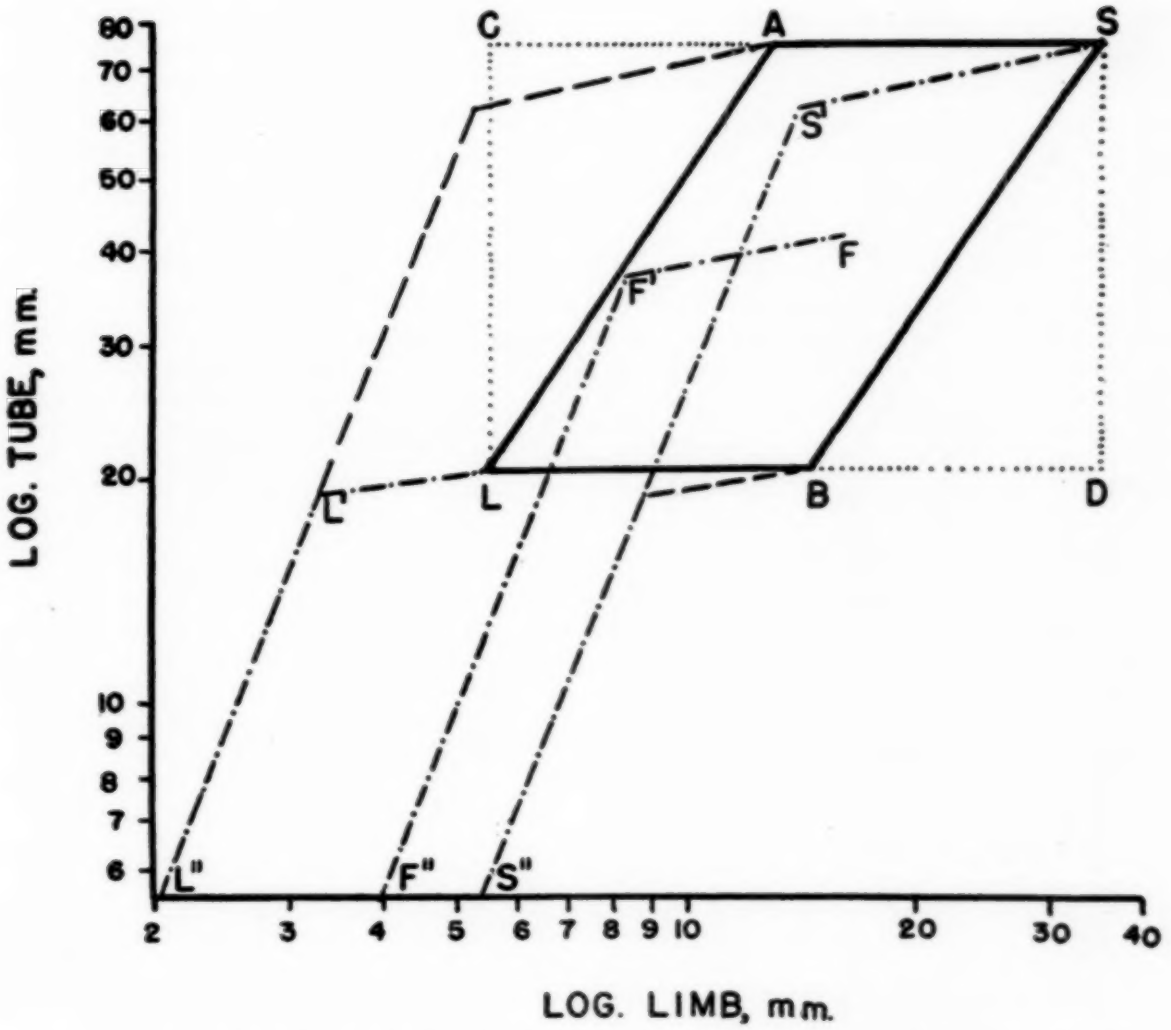

FIG. 3. Relative growth curves for corollas of *N. Langsdorffii* (L"L'L), F_1 (F"F'F) and *N. Sanderae* (S"S'S). The dimensional recombination area is LCSD. The developmental recombination area is LASB.

point some difference in specific relative growth rates was observed, but this factor was of minor importance in determining ultimate dimensions. The developmental analysis has provided us with further insight into the nature of the interspecific differences responsible for corolla size and shape. The F_1 was intermediate, though somewhat nearer to *Sanderae*, in characteristics of relative growth by which the parents differed.

Critical points on curves of corolla development in twenty representative F_2's are shown in table 1. The slopes from bud stage to turning point ranged from 2.2 to 2.8 which was within the limits of error of chance sampling. It was concluded that there was no segregation for this developmental characteristic; it does not constitute a hereditary *difference* between the two species.

There was segregation for the two main characteristics of the relative growth curves by which the parents differed. Limb length at bud stage in the F_2's varied from 2.50 mm. (49–34, table 1) to 4.80 mm. (39–36). Tube length at the turning point varied from 23 to 47 mm. Developmental curves of four most representative F_2 segregants are shown in figure 4. Segregation for early-established shape differences is shown by the variation in proportion of limb to tube length at bud stage; the parental limits being represented as L" and S" on the abscissa. Segregation for amount of elongation be-

TABLE 1. *Critical points on relative growth curves of P_1, F_1 and F_2 corollas and slope of each curve during the major period of growth. F_2's are listed in order of increasing size of mature corolla*

Type	Bud		Slope	"Turning Point"		Mature	
	Tube mm.	Limb mm.	Bud to "T.P."	Tube mm.	Limb mm.	Tube mm.	Limb mm.
N. Langsdorffii	5.50	2.05	2.6	19.00	3.30	20.50	5.50
N. Sanderae	5.50	5.40	2.5	62.00	14.00	74.00	32.00
F_1 *Langs.* × *Sand.*	5.50	3.95	2.6	37.00	8.30	42.00	16.00
F_2—49–34*	5.50	2.50	2.4	23.00	4.50	26.50	8.00
42–18	5.50	3.03	2.3	26.50	6.00	29.00	9.00
39–21	5.50	3.20	2.5	27.00	6.00	30.00	10.00
49–28	5.50	3.12	2.5	26.50	5.90	30.00	10.00
39– 8	5.50	3.80	2.8	28.50	6.80	31.00	12.00
42–75	5.50	3.60	2.5	30.00	7.00	31.50	12.50
37–17	5.50	4.40	2.7	27.50	8.00	32.00	15.00
37–44	5.50	4.10	2.5	28.00	7.80	34.00	14.50
37–64	5.50	3.40	2.3	34.00	7.50	36.00	13.50
49–57	5.50	2.55	2.2	36.50	6.00	40.00	10.00
39–36**	5.50	4.80	2.4	28.00	9.50	34.00	17.00
37– 8	5.50	3.50	2.3	36.00	8.00	37.50	14.00
39– 3**	5.50	2.97	2.5	38.00	6.53	41.00	11.00
37–48	5.50	3.80	2.3	34.00	8.40	40.00	14.00
42–30	5.50	3.70	2.8	40.00	7.60	43.00	15.00
42–40	5.50	3.70	2.4	39.00	8.40	41.00	19.00
39–42	5.50	3.85	2.2	46.00	10.00	49.00	16.00
37– 6	5.50	4.30	2.4	45.50	10.30	52.00	19.50
42–39*	5.50	4.00	2.5	47.00	9.60	52.00	20.00
37–29	5.50	4.40	2.5	44.00	10.00	55.00	20.00

* Relative growth curves shown in figure 4 (a and d).
** Relative growth curves shown in figure 4 (b and c) and corollas illustrated in figure 1 (b and c).

fore the turning point is reached can also be observed in the F_2 curves shown in figure 4. L′ and S′ on the ordinate represent the tube lengths of *Langsdorffii* and of *Sanderae* at this point. The slopes and the relative growth from turning point to maturity are essentially the same in all four F_2 curves.

If recombination of the dimensions tube length and limb length occurred in the F_2, then, in the absence of any restrictions, the expected area of recombination would be LCSD as shown in figure 3. Points L and S are the parental combinations; C represents an F_2 recombination with tube of *Sanderae* and limb of *Langsdorffii;* and D is the other extreme recombination, namely, tube of *Langsdorffii* with limb of *Sanderae*. Such extreme recombination types are not found, or even approached, among these F_2 segregants just as Anderson has repeatedly pointed out for the cross *Langsdorffii* × *alata*. The variability among F_2 corollas is illustrated in figure 1: a, b, c and d.

The developmental observations showed that recombination of independent dimensions is not to be expected, for tube length and limb length maintain a definite relationship to each other during the ontogeny of the corolla. There is a developmental restriction to recombination. In order to estimate the magnitude and nature of this restriction an area of recombination, based on relative growth curves of the species, was constructed. In the absence of transgressive segregation and within the limits of this study, one extreme recombination would be an F_2 in which the relation of tube to limb length in the bud was as in *Langsdorffii* (L″, fig. 3) and subsequent development (from L′ to maturity) followed the pattern of *Sanderae*. This would result in an F_2

recombination type represented at point A in figure 3. The other extreme would be a segregant with bud relationships characteristic of *Sanderae* (S″) combined with the subsequent growth pattern of *Langsdorffii*. This would result in a mature F_2 corolla represented at point B in figure 3.

The area of possible F_2 recombination, constructed by projecting the relative growth curves, is the parallelogram LASB. This area is 53 per cent of that of the dimensional recombination area, LCSD; and the restriction is such as to preclude the possibility of obtaining extreme recombinations in the direction of C and D.

Having established a developmental recombination area it was of interest to plot data on mature F_2 corollas to see where they would fall in relation to this area and what part of it would be covered. Measurements on 931 F_2's from eight yearly plantings were available for plotting. The result is shown in figure 5. The points of the scatter diagram all fell within the area and were rather well centralized, i.e., there was no pronounced restriction in any one direction. An area of observed recombination was enclosed by connecting the outermost points of the F_2 scatter diagram and was calculated to be approximately 40 per cent of the developmental recombination area.

A developmental recombination parallelogram for the backcross to *Langsdorffii* was constructed by using differentiating characteristics of the relative

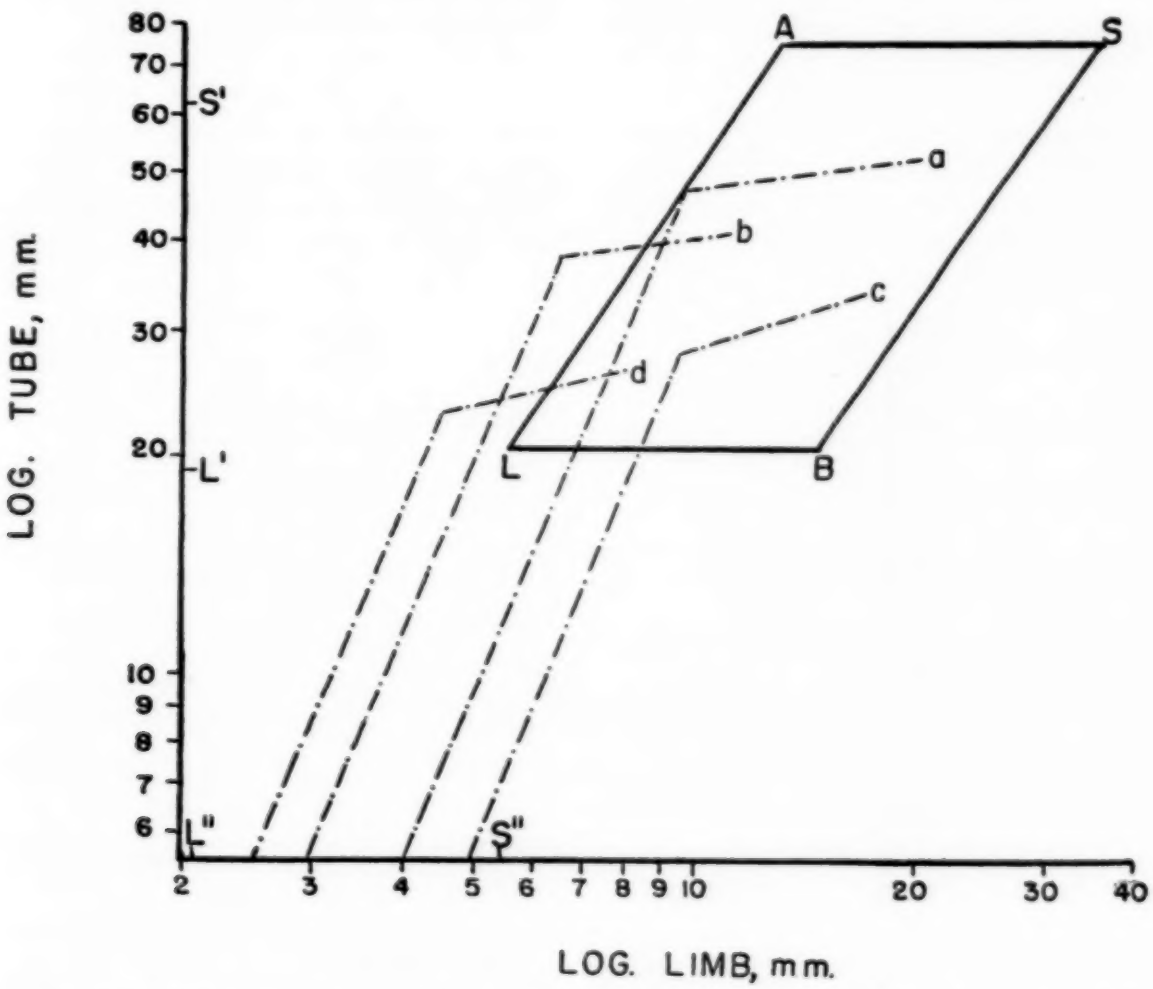

FIG. 4. Relative growth curves for corollas of four representative F_2's. Curves a and d are for relatively large and small segregants, respectively. Curves b and c are for the F_2's whose mature corollas are similarly labeled in figure 1. LASB is the developmental recombination area.

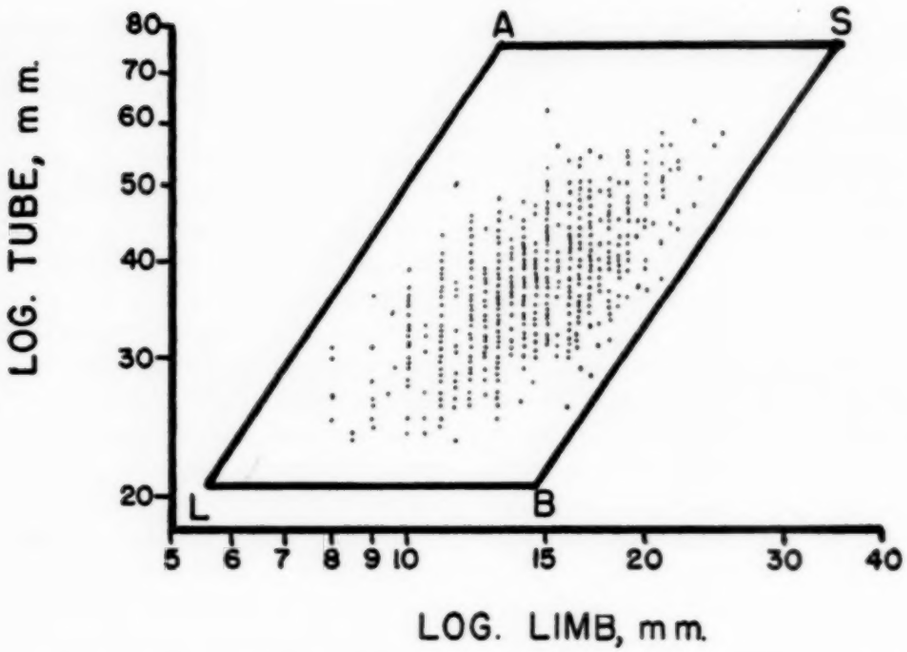

FIG. 5. Scatter diagram from plotting measurements on mature corollas of 931 F_2 segregants. LASB is the developmental recombination area for the F_2.

growth curves of the F_1 and *Langsdorffii* as limits. This area is shown in figure 6. It is approximately 36 per cent that of the F_2 parallelogram LASB. Data on mature corollas of 500 backcross plants from six replications were plotted and the distribution of observed values compared with theoretical expectation. Most points

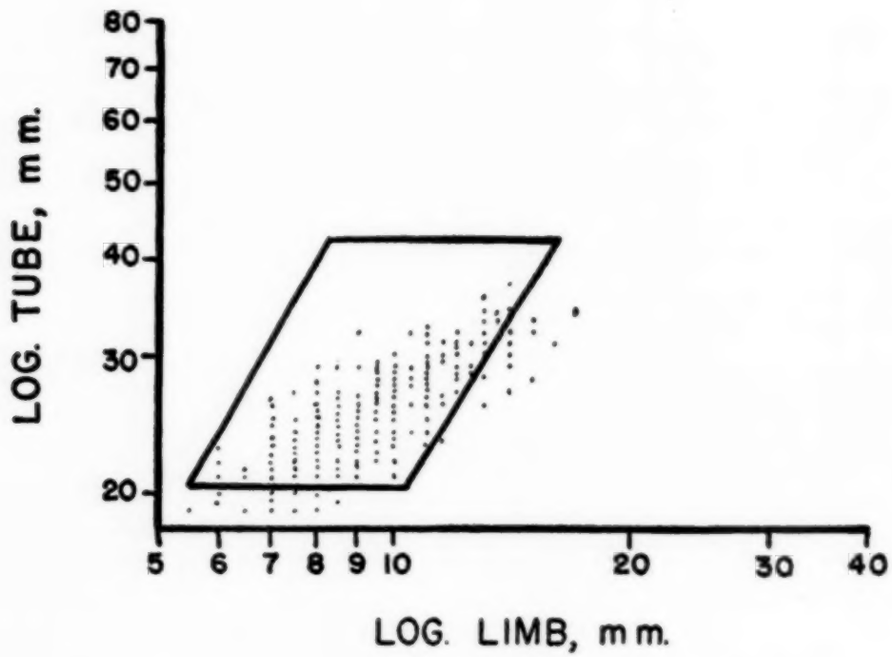

FIG. 6. Developmental recombination area for the backcross to *N. Langsdorffii* and scatter diagram from plotting measurements on mature corollas of 500 backcross plants.

fell within the area. Types nearer to *Langsdorffii* were recovered somewhat more frequently than those nearer the F_1, but the distribution overlapped the range of variability of both. There was a trend among the larger-flowered individuals for a relatively longer limb in proportion to tube than would be predicted on the basis of developmental relationships. This may be significant, and is possibly a consequence of cytological irregularities in the F_1.

A developmental recombination area for the backcross to *Sanderae* was constructed by using the relative growth curve characteristics of the F_1 and *Sanderae* (fig. 7). The area is only 15 per cent as large as the F_2 parallelogram LASB. A scatter diagram was plotted with data on mature corollas of 525 backcross plants from seven replications. Most points fell within the recombination area, while some were distributed outside along each margin. The transgressive segregants were probably due partly to environmental effects, for the theoretical areas were constructed from average values without correction for non-genetic variability, and partly to additional genetic complexities discussed below.

Discussion

Use of relative growth constants has been of value in elucidating problems in evolution (Robb, 1935) and plant morphogenesis (Sinnott, 1937).

The developmental restriction on recombination of corolla tube length and limb length described in this paper is dependent on the fact that *N. Langsdorffii* and *N. Sanderae* have in common a constant relationship of these dimensions to each other during the major period of ontogeny of the organ. This correlation is apparently controlled by the same hereditary factors in both species since segregation for different slopes was not found in the F_2. Dimensional recombination is, therefore, not to be expected.

From the data available, it was considered that only a rough approximation of the developmental recombination areas and the actual genetic variability was made. Other factors would need to be

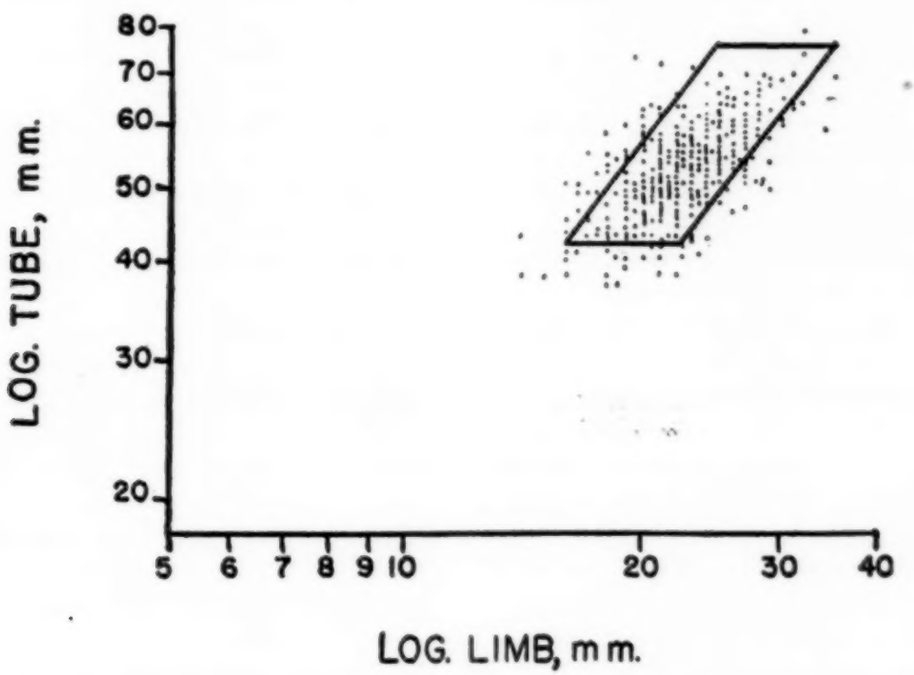

FIG. 7. Developmental recombination area for the backcross to *N. Sanderae* and scatter diagram from plotting measurements on mature corollas of 525 backcross plants.

taken into account in order to make a more correct analysis. Two which should be mentioned here are: (1). The developmental recombination area was formed on the assumption that transgressive segregation could not occur. However, evidence from extra chromosomes (Smith, 1943 and unpublished) indicates that the small-flowered parent, *N. Langsdorffii,* contains some genes for increasing corolla size. If these genes are different from those in *N. Sanderae,* transgressive segregation would be theoretically possible and the postulated recombination area should be larger. (2). Environmental influences increase the variation so that the observed area of recombination was larger than would result from strictly genetic character variation. Furthermore, since the theoretical parallelogram was constructed from average values, the observed area was proportionally, as well as absolutely, larger.

Results of the developmental analysis showed that in estimating the magnitude of effect of other hindrances to recombination it would be unnecessary to have to account for the lack of F_2's representing the extremes of dimensional recombination (toward C and D in fig. 3). Although qualifications as to the accuracy in estimating theoretical and proportional observed recombination areas need to be made, it was nevertheless clear that less severe inhibition remained to be explained from causes other than correlated growth.

Genetic evidence for multifactorial control of inheritance of corolla size and shape in Nicotiana has been well-established since the early classical experiments of E. M. East. The writer calculated by use of Castle's formula that not less than twelve effective genetic blocks of factors were involved in differentiating corolla shape (index = 1000 × log. tube + log. limb) between *N. Langsdorffii* and *N. Sanderae* (Smith, 1937). This estimate was not corrected for possible unequal gene and environmental effects, linkage, dominance, genic interaction, non-isodirectional distribution or sampling error. Nevertheless, it appears reasonable to conclude that multifactorial inheritance alone would account for much of the restriction remaining after correction for developmental hinderances was made.

Restrictions due to pleiotropy of segregating genes, linkage and disharmonies causing differential elimination may be superimposed on those considered above, as Anderson (1939) has discussed for the cross *Langsdorffii* × *alata.* Insofar as can be judged from the data available on this material, the writer is inclined to attach primary importance to the effects of similar relative growth patterns and of multifactorial inheritance in restricting recombination.

Relative growth curves for corolla tube length and limb length have been constructed for various other species of Nicotiana including *Debneyi, glauca, glutinosa, paniculata, sylvestris, tomentosa* and *trigonophylla.* The curves showed a great variety of ontogenetic relationships—different bud proportions, different slopes, different amounts of elongation to the turning point and different relative growth characteristics from the turning point to maturity. The developmental similarities of corollas in *Langsdorffii* and *Sanderae* in spite of the markedly different phenotype are in all probability further indication of the phylogenetic proximity of the two species, which have been placed in the same section of the genus by other investigators on cytogenetic and morphological evidence. Restrictions on recombination can be detected only when hybrids are sufficiently fertile to produce adequate numbers of progeny, in which case the parents are usually closely related. Developmental inhibitions to recombination may be of general importance since related species may be expected to have certain ontogenetic patterns in common. Developmental similarities are undoubtedly the manifestation of similar physiological and morphological conditions.

Acknowledgment

Appreciation is expressed to Dr. Edgar Anderson and Dr. E. R. Dempster for critical reading of the manuscript.

Summary

Two differentiating characteristics that distinguish between the species *Nicotiana Langsdorffii* and *N. Sanderae* are length of corolla tube and limb. In the F_2 generation, derived from the interspecific hybrid, there is a severe restriction to recombination of parental characteristics. Developmental studies were made and relative growth curves constructed for tube length and limb length in the species, F_1 and twenty representative F_2's. All the curves had the common characteristic that the slope was the same from bud stage to just before anthesis when elongation of the tube practically ceased and there was a coincidental rapid extension of the limb until maturity was reached. The curves differed mainly in the proportional length of limb to tube that was established in early bud stage and in the amount of elongation that took place before the "turning point."

An area of expected F_2 recombination was constructed from the relative growth curves of the species and by projecting extreme recombinations of their differentiating ontogenetic characteristics. The developmental recombination area was a parallelogram only 53 per cent as large as the dimensional recombination area and so formed as to exclude extreme recombinative types. There is a severe developmental restriction to recombination owing to the correlated growth pattern common to both species and for which segregation did not occur.

A scatter diagram of measurements on mature corollas of 931 F_2 individuals was plotted and all points fell within the developmental parallelogram. Similar plots were made of measurements on mature corollas of plants in backcrosses of the F_1 to each parent. These fitted reasonably well with developmental recombination areas made from relative growth curves of the F_1 and recurrent parent.

The possible general importance of developmental restrictions, their relation to estimating other hindrances to recombination, and the difficulties in determining the exact magnitude of these effects were discussed.

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ADAPTIVE CHROMOSOMAL POLYMORPHISM IN *DROSOPHILA WILLISTONI*[1]

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Introduction

Organic diversity is, in the main, a response of life to diversity in the environment. The process of evolution has brought forth many and varied species of organisms, because a variety of genotypes adapted to the multitude of environments which exist in the world can not be contained within a single interbreeding population. But species are rarely if ever genetically uniform, and much of the intraspecific polymorphism also has adaptive functions.

Populations of many species of *Drosophila* carry variant chromosomal structures due to the occurrence of inversions of blocks of genes in the chromosomes. This variability seemed adaptively fortuitous until seasonal changes in the incidence of chromosomal structures were found in some California populations of *D. pseudoobscura* (Dobzhansky, 1943) and in Russian populations of *D. funebris* (Dubinin and Tiniakov, 1945). Experiments on artificial populations kept in laboratories showed that individuals homozygous and heterozygous for chromosomal structures have different adaptive values (reviews in Dobzhansky, 1947a, b, 1949).

The biological function of the chromosomal polymorphism in *D. pseudoobscura* and *D. funebris* is, in part, adaptation to seasonal changes in the environment. Any species which inhabits a temperate or a cold climate finds itself exposed at different seasons to a succession of sharply different environments. *D. persimilis* (Dobzhansky, 1948; Spiess, 1950) and *D. robusta* (Carson and Stalker, 1947, 1949; Levitan, 1950) show, however, no pronounced seasonal changes in the incidence of chromosomal variants in the populations so far studied. Here the polymorphism is evidently a response to other than seasonal variations in the environment.

It appeared desirable to compare the chromosomal polymorphisms in temperate and in tropical species of *Drosophila*, and particularly in inhabitants of different tropical climates, some of which are seasonally more constant than others. Populations of *D. willistoni* from different bioclimatic zones of Brazil were chosen as test materials. Taken as a whole, this species shows a greater chromosomal polymorphism than any other organism so far studied. The climates of some parts of Brazil are among the seasonally most constant ones in the Western Hemisphere, while in other parts there is alternation of wet and dry seasons.

Material and Technique

The distribution area of *D. willistoni* extends from central Mexico and southern Florida at least to southern Brazil (Patterson and Wagner, 1943; Burla *et al.*, 1949). *D. willistoni* is, by and large, the commonest and most widespread species of *Drosophila* in Brazil. Its natural food consists of many kinds of fermenting fruit and other vegetable materials (Dobzhansky and Pavan, 1950). Samples of natural populations were collected in the regions of Brazil shown on the maps in figure 1 and figure 2. Smaller samples were obtained, through the courtesy of Professor J. A. Moore, from Costa Rica and from Mexico, and Miss Sophie Dobzhansky collected a sample in Trinidad.

[1] Contribution No. 7 of the cooperative research project of the University of São Paulo and Columbia University on genetics and ecology of tropical *Drosophila*.

These samples were brought or sent to the laboratories in São Paulo or in New York for study.

Single females were placed in culture bottles and allowed to produce offspring. When mature larvae appeared, the salivary glands of one or more larvae from each culture were prepared in temporary acetic orcein mounts. Although *D. willistoni* is not first-class material for studies on salivary gland chromosomes, good cells can be found, with some practice, in almost every slide. A slide was usually given a cursory examination under low magnification, in order to pick out the most favorable cell. Then the gene arrangements in the chromosomes were examined with the aid of an oil immersion (× 90) or a correction collar (× 40) objective. For statistical purposes, the gene arrangements were recorded in only a single larva from each culture.

The Chromosomal Complement

D. willistoni has three pairs of chromosomes: metacentric X or Y chromosomes, metacentric second, and acrocentric third chromosomes. In the cells of larval salivary glands, five chromosomal strands radiate from the heterochromatic chromocenter. Two of them correspond to the left and the right limbs of the X chromosome (designated XL and XR), two to the second (II L and II R), and one to the third chromosome (III). A strain from Belem, Pará, Brazil, has been chosen as standard. The disc patterns in the salivary gland chromosomes of the

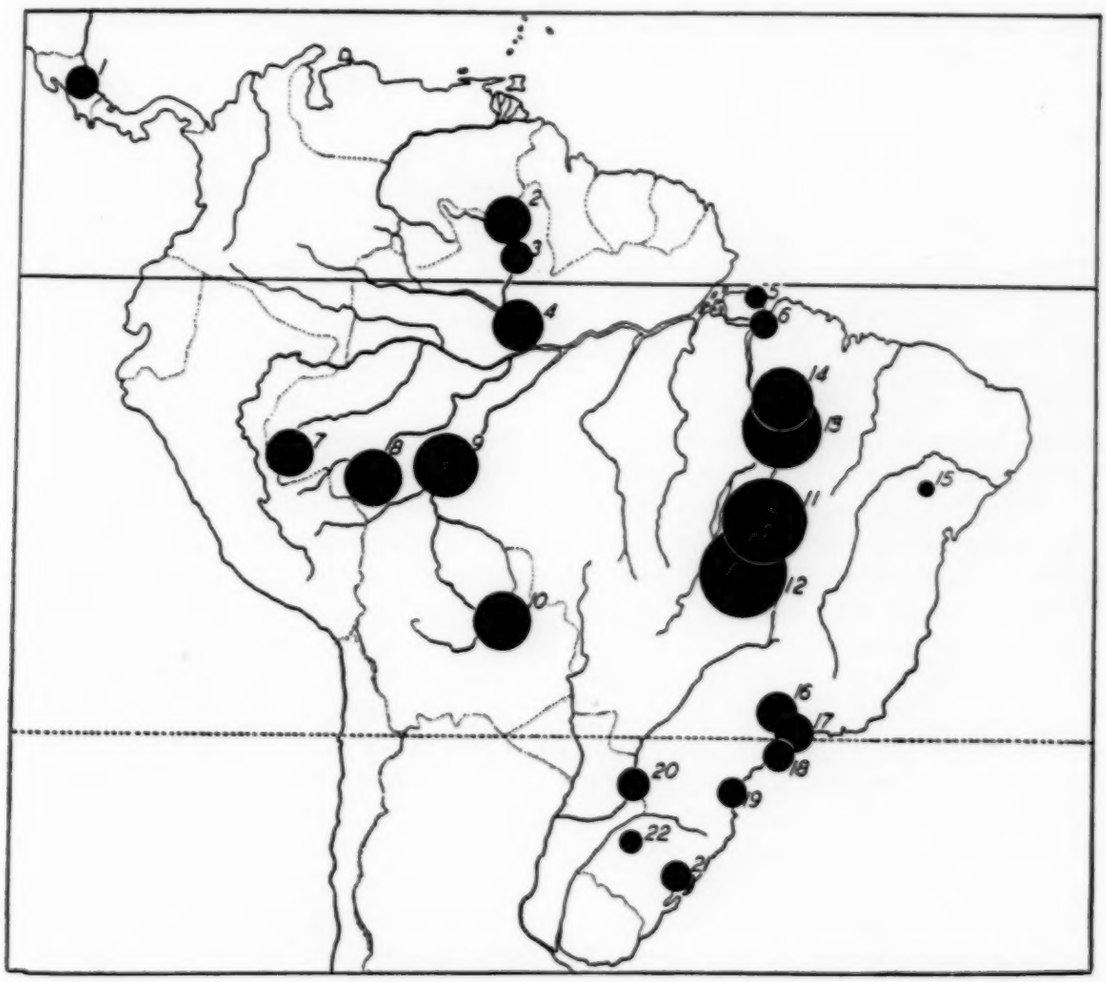


FIG. 1. Average numbers of heterozygous inversions per individual of *Drosophila willistoni* in different regions, symbolized by the diameters of the black circles. Numbers refer to the regions mentioned in tables 3 and 4.

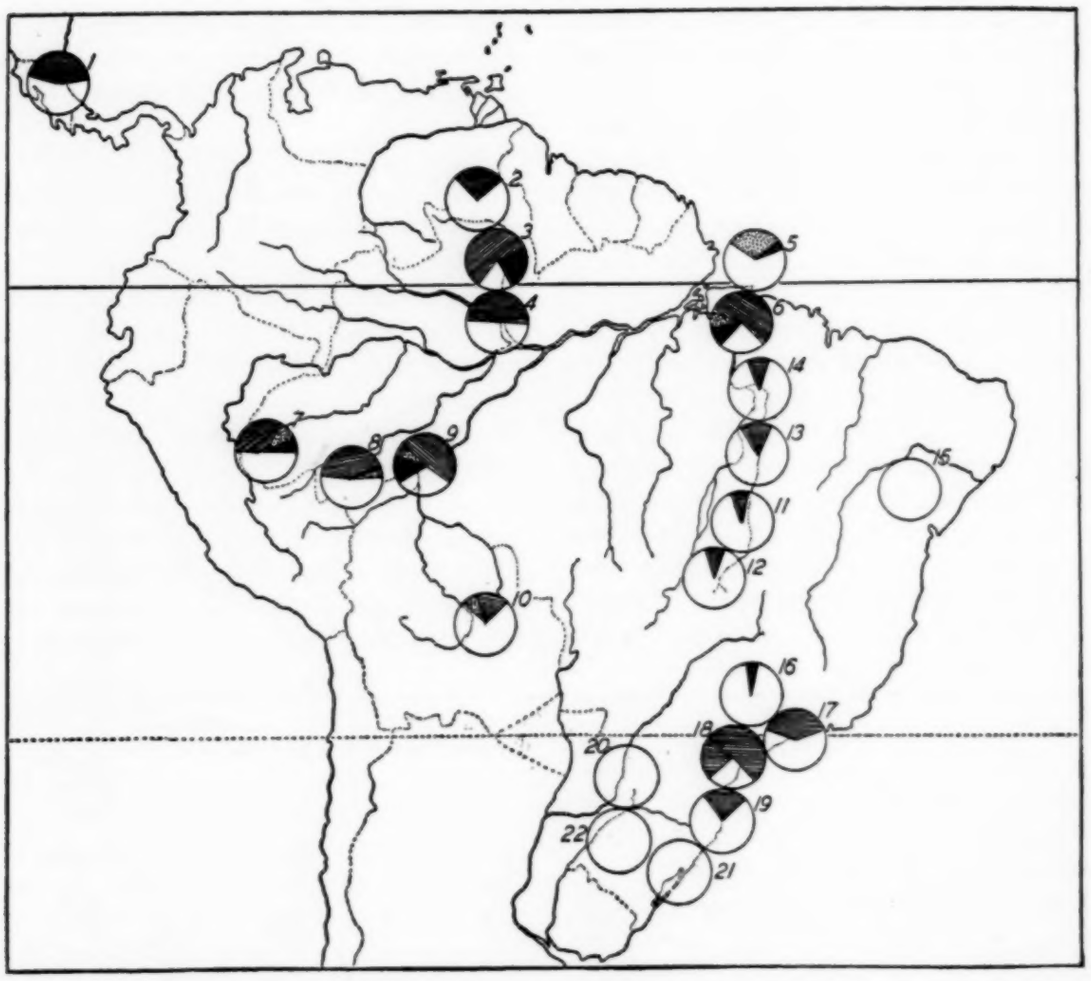


FIG. 2. Relative frequencies of *Drosophila willistoni* (white sectors), *D. paulistorum* (cross-hatched sectors), *D. equinoxialis* (black sectors) and *D. tropicalis* (stippled sectors) in different geographic regions.

standard strain have been drawn in the "maps" published elsewhere (Dobzhansky, 1950b). To facilitate description of the variant gene arrangements, the standard "maps" are divided into 100 arbitrary sections numbered 1–100. The XL strand contains sections 1–16; XR, 17–36; II L, 37–55; II R, 56–77; and III, 78–100.

The variations of gene arrangement known in natural populations of *D. willistoni* are all due to inversions of sections of chromosomes. In some species of *Drosophila*, notably *D. pseudoobscura*, *D. persimilis*, and *D. nebulosa*, the inversions are concentrated in one of the chromosomes, other chromosomes being largely or wholly free of inversions. As many as 40 inversions are known in *D. willistoni*, which is a much greater number than so far found in any other species. These inversions are distributed over all five chromosomal strands, no chromosome being free of inversions. The numbers of inversions known in different chromosomes are as follows:

XL—9	II R—5
XR—5	III—13
II L—8	χ^2—5.500

Although some of the chromosomes seem to be more variable than others, the differences are not significant statistically (probability about 0.25). The uniformity is especially obvious if one takes into account that chromosome III is longer than the rest, and hence should have more in-

versions in it than the shorter chromosomes.

It is further noteworthy that the majority of the inversions in *D. willistoni* are short ones, while in *D. pseudoobscura, D. ananassae, D. robusta, D. melanogaster, D. azteca,* but not in *D. nebulosa,* most inversions include relatively longer sections of the chromosomes. This fact is a source of technical difficulty since the small length of most inversions in *D. willistoni* has made it impractical to distinguish the homozygous standard and inverted gene arrangements. Our data consist, therefore, of records of frequencies of individuals in populations which were heterozygous for a given inversion. Furthermore, since the inversions are short, most of them are independent rather than overlapping or included. (The contrary is true in *D. pseudoobscura* and *D. persimilis,* Dobzhansky and Epling 1944.) Short independent inversions are combined and separated by crossing over, and for this reason the number of combinations of gene arrangements which exist in chromosomes of natural populations of *D. willistoni* is much greater than in any other species of *Drosophila* thus far known. In some samples from central Brazil no two of the hundred or so larvae examined had the same gene arrangement in all chromosomes. This made it impractical to score the frequencies of gene arrangements, and made it necessary to record most of the inversions separately, except some of the overlapping ones. No inversions with breaks in the chromocentral heterochromatin, and no pericentric inversions (including the centromeres), have been found. Their absence indicates that such inversions tend to be discriminated against in natural populations.

Some inversions are predominantly intra-populational, i.e., individuals heterozygous for them occur in many natural populations of the species. Other inversions distinguish geographical populations, i.e., flies of different geographic origin are homozygous for different gene arrangements, and heterozygotes for such inversions occur chiefly in some geographically intermediate populations. Since we recorded chiefly the inversions found in the progeny of wild females fertilized in nature by wild males, our data concern primarily the intra-populational inversions. We also made crosses of strains from different geographic regions to the standard Belem strain (and a similar strain from Marajó Island north of Belem). We do not feel, however, that the geographic distribution of the inversions is well known. A description of the inversions follows.

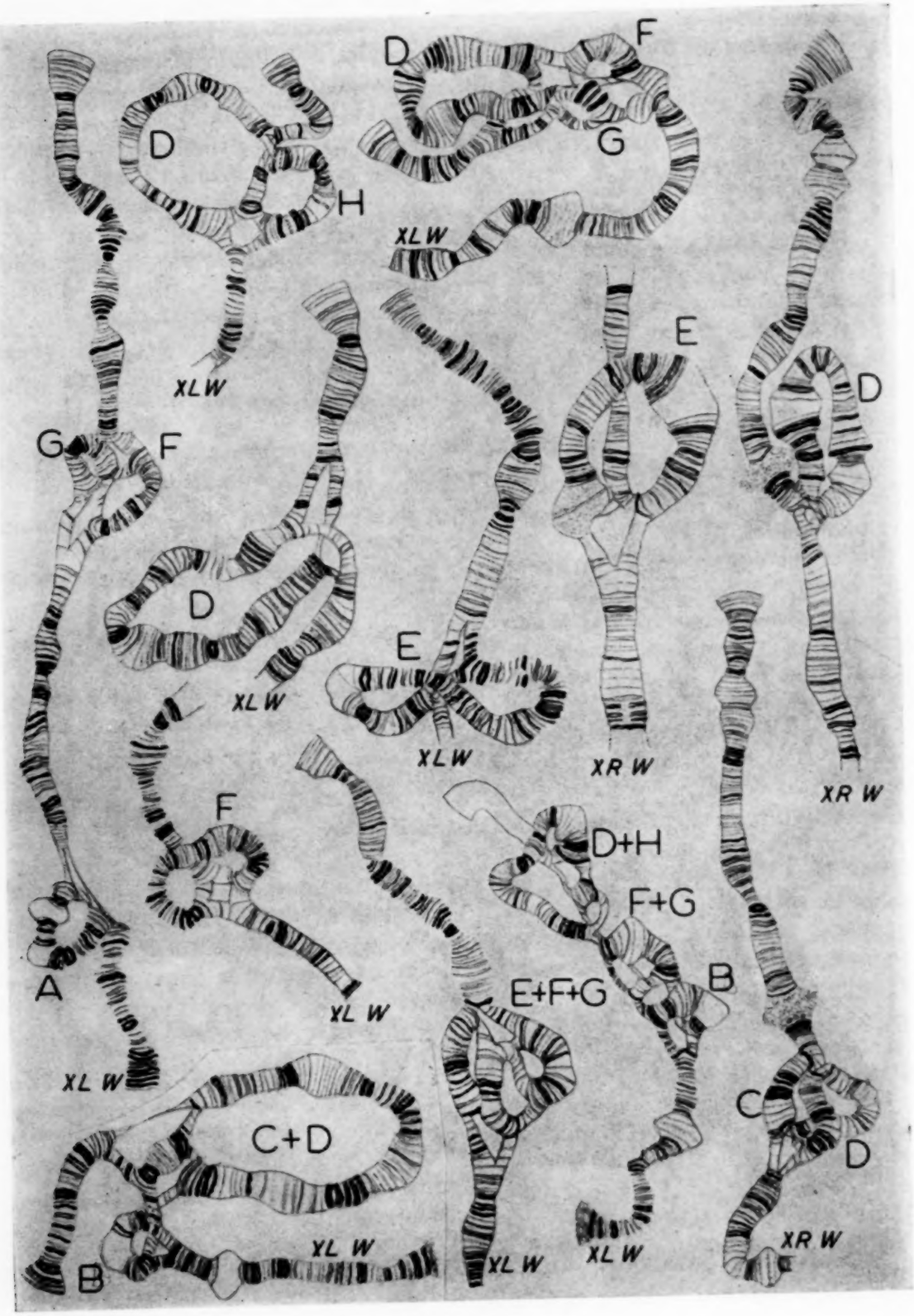
Inversions in the Left Limb of the X Chromosome

XL is the most "difficult" chromosome, owing chiefly to the prevalence of the geographically different gene arrangements. Crosses of the standard strain to strains from southern Brazil usually give the quintuple inversion shown in Plate 1 (B, D, F, G, H), while the triple inversion (B, C, D) is observed in crosses of the standard to Central American (Costa Rica, Guatemala, Mexico) strains. The quintuple and the triple inversions have two elements (B and D) in common, which means that with respect to these two inversions the Central American and the South Brazilian strains are actually more similar than either of them is to the standard strain which is of geographically intermediate origin. The inversions are shown in Plate 1.

Inversion A. Includes sections 3–5 of the standard map. These sections contain two segments which often form light bulbous swellings. Occurs rarely, chiefly in southern Brazil.

Inversion B. Includes the distal part of section 4, section 5, and the proximal part of section 6 of the standard map. Thus, only the distal part of the two "bulbs" is included in this inversion, which overlaps inversion A.

Inversion C. Includes sections from 6 up to the proximal part of 15, and is thus the longest inversion in the species. This inversion has not been seen alone, but it is an integral part of the triple inversion complex which distinguishes the strains from Central America from the standard strain.


D
F
G
H
XLW
D
XLW
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D
XLW
E
XLW
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D
XRW
F
XLW
D+H
F+G
A
E+F+G
B
XLW
XLW
XLW
C+D
XLW
C
D
XRW
B

PLATE 1

Inversion D. Includes sections from the distal part of 7 till the proximal part of 15. The break in section 15 is close to, or identical with, that in inversion C. For this reason, simultaneous heterozygosis for C and D gives the configuration shown in the lower left corner of Plate I. When the short overlapping piece (sections 6 and a part of 7) fails to pair, the chromosome straightens out and shows merely two unpaired intercalations in the subterminal and the subbasal regions, respectively. Inversion D alone occurs in some flies from the Amazon Basin.

Inversion E. Includes sections from the distal portion of 9 to the proximal portion of 15. It has been seen only in a strain from Costa Rica, in which its position is median, since this strain was homozygous for C. Compounds including this inversion have, however, been seen in the Amazon Basin.

Inversion F. Includes sections 9 to 11, and occurs chiefly as an element of multiple inversions in central Brazil. The relations to Inversion E are not clear; the breaks in section 11 are close or even identical.

Inversion G. Includes sections 11 and a part of 7. This inversion is an integral part of the quintuple complex which distinguishes the standard from the south Brazilian strains.

Inversion H. Includes sections 7 to 9. Occurs as a part of the quintuple complex just mentioned, and also singly in south Brazilian strains. Since the latter appear to be homozygous for D, inversion H is located subterminally in the chromosome.

Inversion I. Some strains from southern Brazil contained a double inversion, in which H was certainly one of the elements. The other element was a larger inversion into which I was included, but apparently not quite as long as D. More data are needed to understand the relationships of these three inversions.

Inversions in the Right Limb of the X Chromosome

Some of the inversions known in XR are shown in Plate 1. Most of these inversions occur in the entire distribution area of the species, although the prevalent gene arrangements are different in different geographic regions.

Inversion A. Includes sections 18 to 21. This basal portion of the chromosome, separated from the chromocenter by only a short section No. 17, has two large bulbous swellings, and apparently some interstitial heterochromatin. The pairing in this region, with or without the inversion, is often disturbed, and care must be exercised in order not to mistake spontaneous lack of pairing for an inversion configuration.

Inversion B. Includes sections 18 and 19. The proximal break (in section 18) approximately coincides with that in inversion A, so that B seems to be included in A. Often found together with A.

Inversion C. Includes sections 23–27. Occurs sporadically in populations of central and northern Brazil. Independent of A and B, overlapping D.

Inversion D. Includes sections 23, 24, 25, 28, 29 and the proximal part of 30. Broadly overlaps C. A combination of C and D (see Plate) is characteristic of hybrids between standard and strains from southern Brazil. Inversion D alone is rather common in south Brazilian populations.

Inversion E. Includes sections 30, 29, 28, 23, 24, 25, the distal part of 30 and 31. Easily distinguishable from D because E does, and D does not, include the characteristic "repeat" area of section 31. Since inversion D overlaps C, and E overlaps D, the descent relationships of these inversions can be deduced as follows:

Gene arrangement:	Standard	↔	C	↔	D	↔	E
Distribution:	Amazon Valley		Central Brazil		Southern Brazil		Southern Brazil

Inversions in the Left Limb of the Second Chromosome

The inversions known in II L are shown in Plate 2. Most of them occur widely in the distribution area of the species.

Inversion A. A subbasal inversion including the block of genes from the proximal third of section 38 to the middle of 39.

Explanation of Plates 1–3

Camera lucida drawings of heterozygous inversion configurations in the salivary gland chromosomes of *Drosophila willistoni* (w) and *Drosophila paulistorum* (p). Plate 1—The right (XR) and the left (XL) limbs of the X-chromosome. Plate 2—The right (II R) and the left (II L) limbs of the second chromosome. Plate 3—The third chromosome. The large capital letters designate various inversions described in the text.

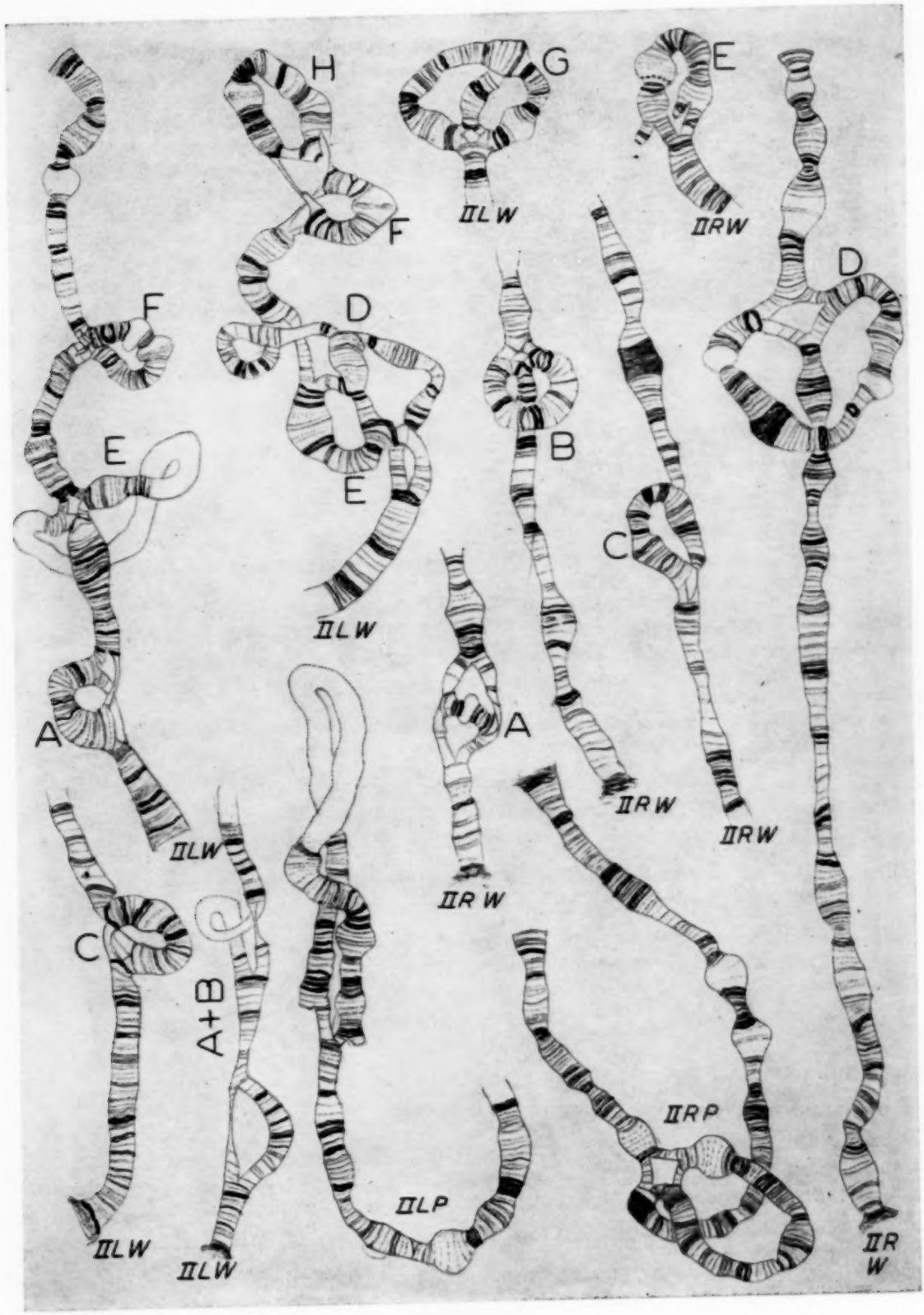
H
G
E
F
IILW
IIRW
D
F
D
B
E
E
C
IILW
A
A
IIRW
IIRW
IILW
IIRW
C
A+B
IIRP
IILP
IILW
IILW
IIR
W

PLATE 2

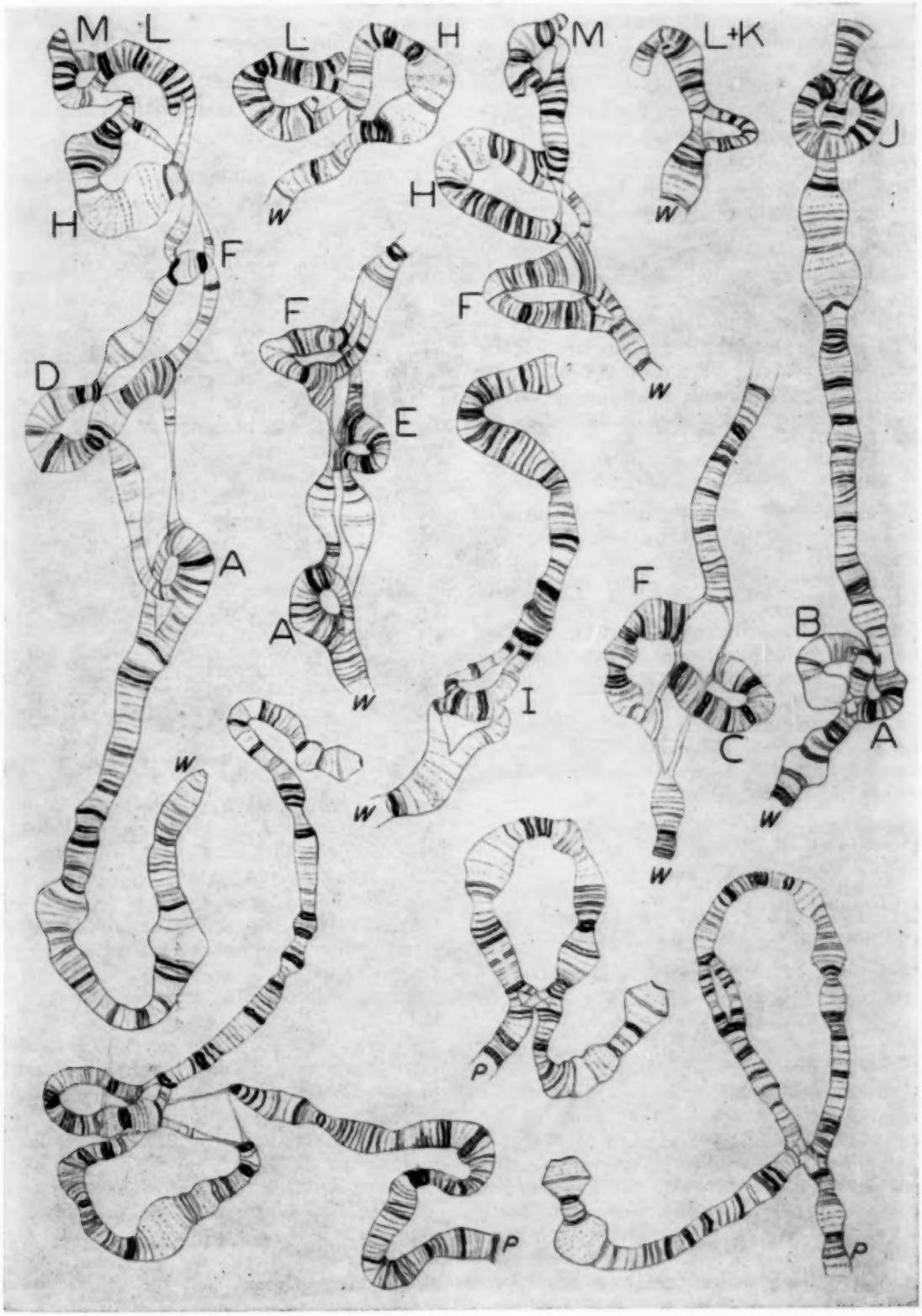

PLATE 3

Inversion B. Includes sections from the middle of 39 to the middle of 40. Broadly overlaps inversion A, and frequently occurs as A/B subbasal double inversion. The drawing of this double inversion in Plate 2 represents a case of incomplete pairing.

Inversion C. Includes sections 40 and 41, overlaps B but not A.

Inversion D. A relatively short median inversion including sections 44, 43, 42 and 48. The inverted gene arrangement differs from standard by two inversions (see Plate 2), the second being inversion E.

Inversion E. A relatively long median inversion which extends from section 42 to 47. Overlaps D, and is often difficult to distinguish from the latter if present alone in a preparation, especially because this part of the chromosome abounds in heterochromatic and repeat areas. The phylogenetic relationships are standard ↔ E ↔ D.

Inversion F. Includes sections 50 to the middle of 52.

Inversion G. A rare subterminal inversion which includes sections 50 to 54 and the proximal part of 55. This gene arrangement is apparently derived from H, thus: standard ↔ H ↔ G.

Inversion H. A relatively short but common subterminal inversion which extends from the middle of 52 up to almost the free end of the chromosome, but not including the few terminal discs which are sometimes paired as shown in Plate 2. The proximal break in H more or less coincides with the distal one in F. Broadly overlaps and almost includes G.

Inversions in the Right Limb of the Second Chromosome

II R is both geographically and individually the least variable chromosome. The five inversions known in it are represented in Plate 2.

Inversion A. A subbasal inversion which includes the distal part of section 57, section 58, and the proximal part of 59.

Inversion B. Includes sections from the distal part of 60 to 62. Independent of A and C.

Inversion C. A median inversion which extends from section 65 to 68. In both terminal sections some groups of discs seem to be partly heterochromatic, which causes irregular pairing in this part of the chromosome in some cells. These irregularly paired chromosomes should not be confused with true inversion heterozygotes.

Inversion D. A rare inversion which includes sections from 67 to 73. It must overlap C, but has never been seen together with the latter.

Inversion E. An apparently terminal inversion, extending from the middle of section 74 to the free end of the chromosome.

Inversions in the Third Chromosome

As many as 13 inversions have been recorded in chromosome III (Plate 3). All inversions are concentrated in the distal two-thirds of the length of the chromosome.

Inversion A. Includes section 87 and the proximal part of 88. This is one of the commonest inversions in southern and central Brazil. It can be seen in three drawings in Plate 3.

Inversion B. Includes sections 88 and 89, thus overlapping inversion A. Although B contains fewer dark discs than A, the two inversions have not been distinguished in the early part of our work. As a consequence, our records show the frequencies of A and B combined, except when both inversions were present simultaneously, in which case they form the configuration shown in Plate 3 (A + B). Inversion B is more common in southern than in northern Brazil.

Inversion C. Includes sections 90 and 91, probably does not overlap B but is included in D. This is a rather rare inversion found in central Brazil.

Inversion D. A relatively long inversion, which extends from section 90 to the basal part of 93, broadly overlaps or includes C and E, and overlaps F. Common in central Brazil but rare or absent in the south.

Inversion E. Includes sections 91 and 92, hence broadly overlaps C and is included in D. Occurs rarely, and forms a double inversion configuration when combined with D which resembles the A + B configuration but can be distinguished from the latter by its more distal position.

Inversion F. Includes section 93 and the distal portion of 92 and the proximal portion of 94. Overlaps slightly inversions E and D. Is shown in four figures in Plate 3. Common in Amazonia and in central Brazil, but is rare in southern Brazil.

Inversion G. Extends from section 94 to 97 inclusive, hence includes H and probably also I, but overlaps F only slightly if at all. G has been seen only in two chromosomes, one from the savanna portion, and the other from the forested portion of the territory of Rio Branco. Unfortunately no good drawing is available.

Inversion H. Includes the distal portion of section 94, 95, 96 and the proximal part of 97. Appears in three figures in Plate 3. Does not overlap F but probably does overlap L slightly.

Very common in most of Brazil, except in the south.

Inversion I. Includes sections 96 and the proximal part of 97, hence is included in H, but probably does not overlap J or L. Rare, but has been found in São Paulo, central Goyaz, and Maranhão. No satisfactory drawing available.

Inversion J. This is probably the commonest inversion in the species, distributed from Costa Rica to Rio Grande do Sul. Includes the distal portion of section 97, 98, 99 and the heavy doublet in the proximal portion of 100. Is included in L and overlaps M.

Inversion K. Found only in two chromosomes from Goyaz, both times in combination with inversion L (Plate 3). Includes parts of sections 98 and 99, and is not derived from the standard gene arrangement but from that modified by L.

Inversion L. Includes sections 97 to 100, except for a few terminal discs in the latter. Slightly overlapping H and I, and probably including J, K and M. Common in most of Brazil, except in the south. Shown in three figures in Plate 3.

Inversion M. Includes the distal portion of the chromosome involved in inversion L, and is apparently derived from the latter. Most often seen as a double subterminal inversion (M + L, Plate 3), and less often by itself (M, Plate 3). The distal break coincides very closely with that in inversion L, making this an almost, but not quite, terminal inversion. Widespread but less common than L.

Temporal Changes in the Chromosomal Constitution of Populations

As stated in the Introduction, cyclic seasonal changes in relative frequencies of inversions occur in certain populations of *D. pseudoobscura* and *D. funebris*. The inversions contain gene complexes which make their carriers better adapted to the environments prevailing at some than at other seasons. During each season, natural selection augments the frequency of the better adapted variants, and reduces that of the less well adapted ones. No seasonal changes occur, however, in *D. persimilis*, *D. robusta*, and in some populations of *D. pseudoobscura*. This certainly does not mean that the seasonally constant inversions are adaptively neutral. Their adaptive significance is presumably related to other than seasonal variations in the habitats, such as different food substances, etc. The adaptive importance of these inversions is indirectly confirmed by the fact that they show not only geographical but also elevational gradients, and in some cases undergo temporal changes not connected with the alternation of seasons (Carson and Stalker, 1947, 1949; Dobzhansky, 1947b, 1948).

Since populations of *D. willistoni* show an unprecedented diversity of gene arrangements, the problem of whether temporal changes occur in this species is especially intriguing. We were able to make periodic collections, at about two month intervals, in three localities in the state of São Paulo. The first locality, Vila Atlantica, is in the rainforest at the foot of the Serra do Mar, some 8 kilometers from the Ocean. Despite its location slightly to the south of the Tropic of Capricorn (lat. 23°50′ south), this locality has a superhumid tropical climate. Monthly temperature means range from 18.6° C. (July) to 25.2° C. (February), and rainfall from 103 mm. (July) to 298 mm. (March) (data for Santos, some 30 km. away). Large populations of *Drosophila* are active throughout the year. The second locality, Mogí das Cruzes, is at an elevation of about 800 m., on the plateau about 77 km. from Vila Atlantica and 36 km. from the coast. Here the winters are too cool and dry (14.7° C. and 30 mm. precipitation in July) for much activity on the part of the flies, but summers are warm and humid (21.5° C. and 208 mm. in January, data for São Paulo, some 50 km. away). The third locality, Pirassununga, is some 213 km. north of Mogí, in the continental interior, with warm but dry winters (18.4° C. and only 14 mm. precipitation in July), and hot and humid summers (23.5° C. and 188 mm. in January).

The observed frequencies of chromosomes with different inversions are summarized in table 1. (Since many chromosomes in natural populations carry more than a single inversion, the sums of

Inversion B. Includes sections from the middle of 39 to the middle of 40. Broadly overlaps inversion A, and frequently occurs as A/B subbasal double inversion. The drawing of this double inversion in Plate 2 represents a case of incomplete pairing.

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Inversion J. This is probably the commonest inversion in the species, distributed from Costa Rica to Rio Grande do Sul. Includes the distal portion of section 97, 98, 99 and the heavy doublet in the proximal portion of 100. Is included in L and overlaps M.

Inversion K. Found only in two chromosomes from Goyaz, both times in combination with inversion L (Plate 3). Includes parts of sections 98 and 99, and is not derived from the standard gene arrangement but from that modified by L.

Inversion L. Includes sections 97 to 100, except for a few terminal discs in the latter. Slightly overlapping H and I, and probably including J, K and M. Common in most of Brazil, except in the south. Shown in three figures in Plate 3.

Inversion M. Includes the distal portion of the chromosome involved in inversion L, and is apparently derived from the latter. Most often seen as a double subterminal inversion (M + L, Plate 3), and less often by itself (M, Plate 3). The distal break coincides very closely with that in inversion L, making this an almost, but not quite, terminal inversion. Widespread but less common than L.

Temporal Changes in the Chromosomal Constitution of Populations

As stated in the Introduction, cyclic seasonal changes in relative frequencies of inversions occur in certain populations of *D. pseudoobscura* and *D. funebris*. The inversions contain gene complexes which make their carriers better adapted to the environments prevailing at some than at other seasons. During each season, natural selection augments the frequency of the better adapted variants, and reduces that of the less well adapted ones. No seasonal changes occur, however, in *D. persimilis*, *D. robusta*, and in some populations of *D. pseudoobscura*. This certainly does not mean that the seasonally constant inversions are adaptively neutral. Their adaptive significance is presumably related to other than seasonal variations in the habitats, such as different food substances, etc. The adaptive importance of these inversions is indirectly confirmed by the fact that they show not only geographical but also elevational gradients, and in some cases undergo temporal changes not connected with the alternation of seasons (Carson and Stalker, 1947, 1949; Dobzhansky, 1947b, 1948).

Since populations of *D. willistoni* show an unprecedented diversity of gene arrangements, the problem of whether temporal changes occur in this species is especially intriguing. We were able to make periodic collections, at about two month intervals, in three localities in the state of São Paulo. The first locality, Vila Atlantica, is in the rainforest at the foot of the Serra do Mar, some 8 kilometers from the Ocean. Despite its location slightly to the south of the Tropic of Capricorn (lat. 23°50′ south), this locality has a superhumid tropical climate. Monthly temperature means range from 18.6° C. (July) to 25.2° C. (February), and rainfall from 103 mm. (July) to 298 mm. (March) (data for Santos, some 30 km. away). Large populations of *Drosophila* are active throughout the year. The second locality, Mogí das Cruzes, is at an elevation of about 800 m., on the plateau about 77 km. from Vila Atlantica and 36 km. from the coast. Here the winters are too cool and dry (14.7° C. and 30 mm. precipitation in July) for much activity on the part of the flies, but summers are warm and humid (21.5° C. and 208 mm. in January, data for São Paulo, some 50 km. away). The third locality, Pirassununga, is some 213 km. north of Mogí, in the continental interior, with warm but dry winters (18.4° C. and only 14 mm. precipitation in July), and hot and humid summers (23.5° C. and 188 mm. in January).

The observed frequencies of chromosomes with different inversions are summarized in table 1. (Since many chromosomes in natural populations carry more than a single inversion, the sums of

TABLE 1. *Frequencies (in per cent) of inversions, and numbers of chromosomes studied, in the state of São Paulo*

Inversion \ Date	Vila Atlantica						Mogí						Pirassununga						
	Sept. 26	Nov. 20	Jan. 10	March 16	May 28	Total	Sept. 4–19	Oct. 23	Dec. 17	Febr. 20	April 19	Total	Sept. 7	Oct. 13–19	Dec. 8–9	Febr. 10	April 8	June 14	Total
XL Chromosome																			
None	83.3	84.0	75.5	64.5	70.4	74.4	65.2	70.0	41.9	64.5	65.1	61.9	72.7	64.1	69.1	53.5	70.8	76.1	67.0
A *or* B	8.3	—	1.9	1.6	7.4	3.7	4.3	—	7.0	3.2	—	2.9	3.0	2.6	5.9	2.8	—	1.5	2.6
F	—	—	—	—	—	—	—	—	7.0	—	—	1.2	6.1	—	—	—	3.1	—	1.0
H	16.7	16.0	20.8	29.0	25.9	22.3	28.3	30.0	55.8	29.0	32.6	34.4	15.2	28.2	26.5	33.8	21.5	20.9	25.4
H + I	—	—	1.9	3.2	—	1.4	2.2	—	—	3.2	2.3	1.2	6.1	6.4	1.5	8.5	4.6	1.5	4.7
D + F + G	—	—	—	1.6	—	0.5	—	—	—	—	1.6	0.4	—	—	—	—	—	—	—
Chromosomes studied	48	25	53	62	27	215	46	50	43	62	43	244	33	78	68	71	65	67	382
XR Chromosome																			
None	85.4	76.0	75.5	69.4	70.4	75.3	63.0	90.0	51.2	58.1	46.5	62.3	81.8	76.9	80.9	66.2	72.3	80.6	75.9
A	—	—	—	1.6	3.7	0.9	—	2.0	9.3	9.7	—	4.5	—	—	1.5	1.4	—	1.5	0.8
A + B	—	—	—	—	—	—	—	—	7.0	—	—	1.2	—	—	—	—	1.5	—	0.3
D	14.6	20.0	24.5	24.2	25.9	21.9	23.9	8.0	37.2	22.6	44.2	26.2	12.1	17.9	16.2	28.2	18.5	16.4	18.8
E	—	4.0	—	4.8	—	1.9	13.0	2.0	9.3	9.7	9.3	8.2	6.1	6.4	1.5	4.2	7.7	1.5	4.4
Chromosomes studied	48	25	53	62	27	215	46	50	43	62	43	244	33	78	68	71	65	67	382
II L Chromosome																			
None	22.7	29.7	28.8	25.0	25.6	26.1	13.8	16.4	20.6	12.0	14.7	15.2	20.9	8.2	17.1	13.0	11.0	11.0	12.7
A	9.1	2.7	5.0	9.0	9.3	7.4	8.6	—	11.1	18.0	17.3	11.8	20.9	24.5	23.8	20.0	23.1	20.0	22.2
C	15.2	13.5	8.8	13.0	18.6	13.2	32.8	13.4	14.3	18.0	16.0	18.5	18.6	16.4	19.0	11.0	7.7	23.0	15.8
D	40.9	35.1	33.8	27.0	23.3	31.9	51.7	40.3	36.5	33.0	42.7	39.9	53.5	58.2	33.3	42.0	38.5	40.0	43.5
E	33.3	13.5	15.0	35.0	27.9	26.4	27.6	32.8	23.8	24.0	22.7	25.9	37.2	52.7	38.1	27.0	25.3	26.0	34.6
F	40.9	45.9	47.5	39.0	41.9	42.6	43.1	32.8	44.4	47.0	56.0	45.2	41.9	47.3	46.7	45.0	49.5	59.0	48.8
H	7.6	8.1	3.8	12.0	7.0	8.0	6.9	6.0	9.5	10.0	5.3	7.7	2.3	13.6	8.6	10.0	16.5	15.0	11.8
Chromosomes studied	66	37	80	100	43	326	58	67	63	100	75	363	43	110	105	100	91	100	549

TABLE 1.—*Continued*

Inversion \ Date	Vila Atlantica						Mogí						Pirassununga –						
	Sept. 26	Nov. 20	Jan. 10	March 16	May 28	Totai	Sept. 4–19	Oct. 23	Dec. 17	Febr. 20	April 19	Total	Sept. 7	Oct. 13–19	Dec. 8–9	Febr. 10	April 8	June 14	Total
II R Chromosome																			
None	87.9	100.0	95.0	87.0	93.0	91.4	89.7	94.0	85.7	78.0	90.7	86.5	93.0	87.3	81.9	87.0	84.6	90.0	86.5
A	1.5	—	—	—	—	0.3	—	1.5	1.6	2.0	—	0.6	—	1.8	1.0	1.0	—	1.0	0.5
B	—	—	—	—	—	—	—	—	1.6	2.0	—	0.8	—	2.7	1.9	1.0	—	1.0	0.9
C	1.5	—	—	2.0	—	0.9	—	—	4.8	3.0	—	1.7	—	—	—	—	—	—	—
D	—	—	—	—	—	—	—	—	—	—	1.3	0.3	—	—	—	—	—	—	—
E	9.1	—	5.0	11.0	7.0	7.4	10.3	4.5	6.3	15.0	8.0	9.4	7.0	8.2	16.2	11.0	15.4	8.0	11.3
Chromosomes studied	66	37	80	100	43	326	58	67	63	100	75	363	43	110	105	110	91	100	549
Third Chromosome																			
None	22.7	29.7	23.8	17.0	39.5	24.2	25.9	26.9	31.7	18.0	25.3	24.8	30.2	11.8	15.2	14.0	12.1	16.0	15.1
A *or* B	51.5	59.5	60.0	54.0	41.9	54.0	50.0	37.3	54.0	53.0	46.7	48.5	32.6	47.3	63.8	60.0	60.4	51.0	54.5
A + B	9.1	—	1.2	8.0	2.3	4.9	1.7	3.0	1.6	14.0	13.3	7.7	16.3	14.5	7.6	12.0	12.1	12.0	12.0
D	—	—	—	—	—	—	—	—	—	3.0	1.3	1.1	—	0.9	—	3.0	8.8	1.0	2.4
E + F	—	—	—	—	—	—	—	—	—	—	—	—	—	3.6	—	—	—	3.0	1.2
F	—	—	—	2.0	2.3	0.9	3.4	—	3.2	8.0	4.0	4.1	2.3	6.4	3.8	6.0	5.5	4.0	4.9
H	—	2.7	—	7.0	2.3	2.8	1.7	1.5	3.2	9.0	6.7	5.0	7.0	1.8	11.4	13.0	16.5	6.0	9.3
J	43.9	32.4	38.7	41.0	41.9	40.2	46.6	52.2	44.4	50.0	48.0	48.5	39.6	43.6	46.7	43.0	41.8	41.0	43.0
L	—	—	—	1.0	—	0.3	—	—	—	—	1.3	0.3	—	—	1.9	2.0	—	—	0.7
M	—	—	—	—	—	—	—	—	1.6	—	—	0.3	—	—	—	—	—	—	—
L + M	—	2.7	—	4.0	2.3	1.8	1.7	1.5	3.2	8.0	5.3	4.4	7.0	14.6	3.8	11.0	14.3	5.0	9.5
Chromosomes studied	66	37	80	100	43	326	58	67	63	100	75	363	43	110	105	100	91	100	549

TABLE 2. *Degree of homogeneity of the frequencies of certain inversions at different seasons*

Chromosome and inversion	Vila Atlantica		Mogí		Pirassununga	
	x^2	P	x^2	P	x^2	P
XL, Inv. H	2.60	0.6	7.08	0.15	4.53	0.5
XR, Inv. D	1.73	0.8	14.02	0.008	4.58	0.5
II L, Inv. C	2.35	0.7	10.07	0.04	9.43	0.09
II L, Inv. F	0.92	0.9	4.34	0.35	3.01	0.7
II L, Inv. H	3.89	0.4	1.80	0.75	7.32	0.2
II R, Inv. E	5.39	0.25	5.92	0.2	6.20	0.3
III, A or B	1.98	0.75	2.61	0.6	10.26	0.07
III, A + B	9.62	0.05	15.93	0.003	2.92	0.7
III, J	0.87	0.9	0.50	0.95	0.60	0.95
III, H	—	—	—	—	15.10	0.01
III, L + M	—	—	—	—	11.40	0.04
Degrees of freedom	4		4		5	

the percentages of all inversions in a sample are often greater than 100 per cent.) In all, 32 different inversions (out of the 40 known in the species) occur in the three localities under consideration. Some of them are common, and others rare. The observed frequencies of the inversions fluctuate in the different samples taken in the same locality in different months. The statistical significance of these fluctuations can be evaluated by computing chi-squares for homogeneity. Such computations have been made for all inversions which are frequent enough in the samples to make the application of the chi-square method free of difficulties. (No tests were made for inversions D and E in chromosome II L because these inversions are not easily distinguishable and their recorded frequencies may be inaccurate.) The results are summarized in table 2.

For most of the inversions, the frequencies observed in samples taken at different times in a given locality are alike within the limits of sampling errors. Inversion D in the XR chromosome at Mogí, A + B in the third chromosome at Mogí, and H and perhaps L + M in the third chromosome at Pirassununga are exceptions because the frequencies of these inversions varied significantly during the period of observation.

Since our observations extended only from September, 1948, to June, 1949, we do not know whether the observed changes in the frequencies of the inversions are a part of a seasonal cycle, or whether these changes reflect other than seasonal alterations in the environment. Both seasonal and non-seasonal changes have been observed in *D. pseudoobscura* and *D. robusta*. As an attempt to get additional information bearing on this point, the data in Table 1 have been re-calculated by summing up, for each locality and inversion, the records for the hot months (December–March), and for the cooler months (September–November and April–June). Chi-squares, having each one degree of freedom, were then computed for these hot season-cool season comparisons. If the frequencies of some inversions varied seasonally one could expect that they would be different during climatically most distinct seasons. In reality, not one of the 31 chi-squares came up to the 5 per cent probability level, not even for those inversions which gave significant heterogeneities in comparisons of individual samples. This suggests, although it does not prove, that the observed changes are not seasonal.

It is commonplace that seasonal changes in the purely physical parameters of the environment (temperature, length of day,

etc.) are smaller in the tropics than in the temperate zones, and some tropical climates, such as that of Vila Atlantica, are more constant than others, such as that of Pirassununga. The indicated absence of seasonal changes in the frequencies of inversions may, then, seem trivial. In reality, the environment is far from seasonally constant even at Vila Atlantica because of changes in biotic parameters, such as the availability of different species of fruits on which the flies feed. Dobzhansky and Pavan (1950) have shown that the relative frequencies of the 30 to 35 species of Drosophila which occur at Vila Atlantica, Mogí, and Pirassununga, undergo changes from month to month, which are at least as great as the changes recorded by Patterson (1943) for species of *Drosophila* in Texas. It is, however, an open question whether the changes in the relative abundance observed in the Brazilian localities are cyclic and seasonal. Some of the data of Dobzhansky and Pavan indicated that they are not regularly seasonal, and Dr. Pavan kindly informs us that his most recent observations tend in the same direction. When as many as 30 ecologically fairly similar species compete for a group of kindred habitats in the same locality, the biotic system is highly complex. Now, the environment varies not only from season to season but also from year to year, and, in fact, a given constellation of physical and biotic variables is never repeated. This makes regular cycles either in the relative abundance of species or in relative frequencies of inversions improbable in regions where many species share the same habitat.

The three localities in which periodic sampling has been done are in the state of São Paulo, at the southern margin of the tropical zone. It is, therefore, interesting that temporal variations in the relative abundance of *Drosophila* species occur also in the equatorial climate of Belem do Pará, where samples in the same neighborhood were taken in June, 1948, and in May and July–September, 1949 (Dobzhansky and Pavan, 1950). Analysis of the chromosomes of *D. willistoni* was made in the samples of May and of July–September, 1949. Despite the small number of individuals analyzed (77 and 78, respectively), one of the inversions, namely F in the third chromosome, proved to be significantly more frequent in the May than in the July–September samples ($\chi^2 = 7.08$, with 1 degree of freedom, $P = 0.008$). The reverse situation obtained for the subbasal inversions (A and B) in II L chromosome, which were less frequent in May than in July–September ($\chi^2 = 8.91$).

Indication of Adaptive Superiority of Inversion Heterozygotes

Apart from the seasonal changes in the frequency of inversions, the evidence that the inversions found in natural populations are adaptively important comes from two sources. First, experiments on artificial populations kept in population cages in the laboratory show that, under certain conditions, natural selection modifies the frequencies of the inversions (Wright and Dobzhansky, 1946; Dobzhansky, 1947 for *D. pseudoobscura;* Dubinin and Tiniakov, 1947 for *D. funebris;* Spiess, 1950 for *D. persimilis;* Levitan, 1950 for *D. robusta*). Secondly, in *D. pseudoobscura*, inversion heterozygotes have been shown to be more frequent in nature, compared to homozygotes, than they would be if their viabilities were alike (Dobzhansky and Levene, 1948). Neither type of evidence is yet available for *D. willistoni*, so that our assumption of adaptive importance of the inversions in this species rests almost entirely on analogy with the species which have been studied in this respect. In no species have naturally occurring inversions proven to be adaptively neutral. The only more direct evidence for *D. willistoni* is as follows:

If homozygotes and heterozygotes for an inversion are equally viable between the egg and the adult stage, the highest frequency which the heterozygotes can reach in a panmictic population is 50 per

TABLE 3. *Frequencies (in per cent) of inversions and numbers of chromosomes in different regions*

Inversion \ Region	1. Costa Rica	2. Rio Branco Savanna	3. Rio Branco Forest	4. Rio Negro Amazonas	5. Marajo Island	6. Belem, Para	7. Western Acre	8. Eastern Acre	9. Guapore	10. Santa Cruz Bolivia	11. Palma Goyaz	12. Monjolinho Goyaz	13. Carolina Maranhão	14. Imperatriz Maranhão	15. Caturni, Bahia	16. Pirassununga São Paulo	17. Mogi São Paulo	18. Vila Atlantica São Paulo	19. Paranagua Parana	20. Iguassu Parana	21. Reuter, Rio Grande Sul	22. Santo Angelo Rio Grande Sul
XL Chromosome																						
None	62.5	76.7	72.0	42.4	100.0	96.1	74.7	56.4	61.5	37.5	34.1	25.3	17.6	53.4	72.6	67.0	61.9	74.4	88.8	93.0	100.0	94.7
A *or* B	12.5	2.3	—	9.1	—	3.0	—	—	—	50.0	20.5	42.1	5.9	3.4	1.8	2.6	2.9	3.7	—	2.3	—	—
D	—	—	4.0	9.1	—	1.0	—	—	—	12.5	36.4	30.5	17.6	—	25.7	—	—	—	—	—	—	—
E	37.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F	—	—	—	—	—	1.0	2.7	17.9	23.1	—	—	—	5.9	3.4	—	1.0	1.2	—	—	—	—	—
E + F	—	2.3	—	6.1	—	—	22.7	25.6	15.4	—	—	—	—	—	—	—	—	—	—	—	—	—
D + E	—	9.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
G	—	—	—	—	—	—	—	—	—	—	6.8	8.4	—	—	—	—	—	—	—	—	—	—
F + G	—	—	—	—	—	—	—	—	—	—	6.8	3.6	35.3	3.4	—	—	—	—	—	—	—	—
D + F + G	—	4.6	—	9.1	—	1.0	—	—	—	—	11.4	20.5	29.4	39.6	—	—	0.4	0.5	—	1.2	—	—
H	—	—	—	—	—	—	—	—	—	—	29.5	42.1	5.9	—	—	25.4	34.4	22.3	11.1	3.5	—	5.3
D + H	—	7.0	24.0	36.4	—	—	—	—	—	—	4.6	—	—	—	—	—	—	—	—	—	—	—
H + I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.7	1.2	1.4	—	—	—	—
XR Chromosome																						
None	87.5	65.1	92.0	69.7	73.7	92.2	93.3	87.2	69.3	100.0	29.5	36.8	47.1	65.5	100.0	75.9	62.3	75.3	88.9	96.5	50.0	100.0
A *or* B	—	2.3	4.0	21.2	—	6.9	2.7	5.2	7.7	—	59.1	36.8	—	31.0	—	1.1	5.7	0.9	5.5	1.2	20.0	—
C	—	—	—	—	—	1.0	—	—	—	—	—	6.2	5.9	—	—	—	—	—	—	—	—	—
D	—	2.3	4.0	9.1	—	—	4.0	7.7	23.1	—	27.3	36.8	—	—	—	18.8	26.2	21.9	5.5	—	20.0	—
C + D	12.5	30.2	—	—	26.3	—	—	—	—	—	—	50.0	47.1	3.4	—	—	—	—	—	—	—	—
E	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.4	8.2	1.9	—	3.5	0.0	—
X-chromosomes studied	8	43	25	33	19	102	75	39	13	8	44	83	17	58	113	382	244	215	18	86	10	19
II L Chromosome																						
None	40.0	38.3	72.2	25.8	67.6	33.5	28.3	16.4	5.9	15.4	8.3	7.0	9.4	6.0	82.0	12.7	15.2	26.1	33.3	23.9	40.0	77.8
A *or* B	10.0	17.3	11.1	29.0	17.7	18.7	33.3	35.8	41.2	30.7	40.3	39.1	37.5	22.0	—	22.2	11.8	7.4	20.8	20.9	—	—
A + B	—	22.2	16.7	27.4	11.8	27.1	19.2	11.9	17.6	—	26.4	8.6	21.9	16.0	1.3	—	—	—	—	—	—	—
C	10.0	9.9	—	—	2.9	0.6	—	—	17.6	7.7	6.9	7.8	6.2	2.0	—	15.8	18.5	13.2	16.7	7.5	33.3	3.7
D	—	11.1	—	11.3	—	—	5.1	6.0	29.4	15.4	8.3	47.7	40.6	2.0	—	43.5	39.9	31.9	20.8	37.3	33.3	3.7
E	20.0	1.2	2.8	—	2.9	0.6	11.1	6.0	29.4	15.4	26.4	28.1	12.5	—	15.3	34.6	25.9	26.4	16.7	29.1	13.3	—
F	40.0	17.3	8.3	33.9	17.6	25.8	43.4	50.7	41.2	84.6	54.2	50.0	62.5	51.0	2.7	48.8	45.2	42.6	20.8	22.4	26.6	11.1
G	—	—	—	—	—	—	—	—	—	—	2.8	—	—	—	—	—	—	—	—	—	—	—
H	—	2.5	—	9.7	—	—	3.0	13.4	29.4	7.7	4.2	7.8	3.1	1.0	—	11.8	7.7	8.0	20.8	15.7	—	7.4

TABLE 3.—*Continued*

Inversion \ Region	1. Costa Rica	2. Rio Branco Savanna	3. Rio Branco Forest	4. Rio Negro Amazonas	5. Marajo Island	6. Belem, Para	7. Western Acre	8. Eastern Acre	9. Guapore	10. Santa Cruz Bolivia	11. Palma Goyaz	12. Monjolinho Goyaz	13. Carolina Maranhão	14. Imperatriz Maranhão	15. Catuni, Bahia	16. Pirassununga São Paulo	17. Mogi São Paulo	18. Vila Atlantica São Paulo	19. Paranagua Parana	20. Iguassu Parana	21. Reuter, Rio Grande Sul	22. Santo Angelo Rio Grande Sul
II R Chromosome																						
None	50.0	70.3	94.4	85.5	94.1	93.5	44.4	43.3	64.7	69.2	27.8	31.3	78.1	91.0	89.3	86.5	86.5	91.4	100.0	91.0	93.3	96.3
A	30.0	1.2	—	—	—	—	—	7.5	5.9	—	6.9	4.7	6.3	—	—	0.5	0.6	0.3	—	—	—	—
B	—	4.9	—	—	—	—	23.2	19.4	23.5	—	31.9	19.5	—	1.0	—	0.9	0.8	—	—	0.7	—	—
C	—	6.2	—	—	—	1.3	2.0	6.0	11.8	—	9.7	10.9	9.4	3.0	—	—	1.7	0.9	—	0.7	—	—
D	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.3	—	—	—	—	—
E	30.0	19.8	5.6	14.5	5.9	5.2	43.4	35.8	5.9	30.8	47.2	53.9	9.4	5.0	10.7	11.3	9.4	7.4	—	7.4	6.7	3.7
III Chromosome																						
None	40.0	17.3	27.7	11.3	64.7	32.3	11.1	6.0	5.9	23.1	6.9	3.1	3.1	14.0	80.0	15.1	24.8	24.2	20.8	13.4	26.7	29.6
A *or* B	—	34.6	11.1	25.8	8.8	15.5	58.6	62.7	23.5	53.8	40.3	61.7	59.4	37.0	1.3	54.5	48.5	54.0	37.5	54.5	33.3	37.0
A + B	—	7.4	5.5	1.6	—	1.3	—	1.5	11.8	—	6.9	8.6	—	—	—	12.0	7.7	4.9	20.8	24.6	13.3	14.8
C	—	1.2	—	—	—	12.9	—	—	—	—	2.8	1.6	—	5.0	—	—	—	—	—	—	—	—
D	20.0	27.2	27.7	38.7	17.6	13.5	17.2	17.9	41.2	23.1	16.7	28.9	18.8	45.0	0.7	2.4	1.1	—	—	—	—	—
E	—	3.7	—	—	—	3.2	—	—	—	—	2.8	0.8	—	—	—	1.2	—	—	—	—	—	—
F	20.0	13.3	27.7	33.9	23.5	40.0	41.4	35.8	64.7	15.4	51.4	54.7	37.5	26.0	0.7	6.1	4.1	0.9	—	1.5	—	3.7
G	—	1.2	2.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
H	10.0	29.6	27.7	46.8	—	7.7	57.5	65.7	82.4	61.5	55.6	61.7	65.6	39.0	6.0	9.3	5.0	2.8	—	3.7	—	3.7
I	—	—	—	—	—	—	—	—	—	—	1.4	0.8	9.4	—	—	—	—	—	—	—	—	—
J	20.0	35.8	16.7	25.8	14.7	38.7	5.0	4.5	—	15.4	4.2	5.5	21.9	50.0	13.3	43.0	48.5	40.2	41.7	44.8	40.0	59.3
K	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	—	—	—	—	—	—	—	—
L	30.0	12.3	30.6	29.0	—	0.6	—	—	—	—	15.3	12.5	21.9	—	—	0.7	0.3	0.3	—	—	—	—
M	—	—	—	1.6	—	—	—	—	—	—	15.3	9.4	—	—	—	—	0.3	—	—	—	—	—
L + M	—	9.9	—	1.6	—	0.6	51.5	55.2	41.2	23.1	29.2	44.5	21.9	17.0	0.7	9.5	4.4	1.8	—	1.5	—	—
Autosomes studied	10	81	36	62	34	155	99	67	17	13	72	128	32	100	150	549	363	326	24	134	15	27

cent. This occurs if the two alternative gene arrangements differing in the inversion are equally common, i.e., have gametic frequencies equal to 0.5. Yet, in the population samples studied by ourselves frequencies above 50 per cent are recorded for some inversions (tables 1 and 3). In most cases the excess over 50 per cent is not statistically assured. However, inversion H in the third chromosome showed frequencies higher than 50 per cent in all samples from a large territory which extends from Santa Cruz de la Sierra, in Bolivia, to Acre, Guaporé, Goyaz, and Carolina, in Maranhão (table 3). The deviations from 50 per cent are more than twice as large as their standard errors for Eastern Acre, for Guaporé, and for Monjolinho, in Goyaz.

Geographic Distribution of Inversions

Most of the inversions recorded in the chromosomes of *D. willistoni* are distributed very widely. Although the sample studied from Costa Rica consisted of only 10 flies, and was studied two generations after capture, it contained 11 autosomal inversions, 7 of which occur also throughout Brazil, including the southernmost state of Rio Grande do Sul. The remaining 4 inversions do not seem to reach Rio Grande do Sul, but go at least as far as the state of São Paulo, on the Tropic of Capricorn (cf. table 3 and fig. 1). Vice versa, among the 42 flies studied from Rio Grande do Sul, 11 autosomal inversions were found, every one of which occurs throughout Brazil, including the equatorial Amazonian regions.

A few of the inversions seem to be endemic (XL–E in Costa Rica, XL–I in São Paulo, II L–G in Goyaz, II R–D in São Paulo, III–G in the territory of Rio Branco, III–K in Goyaz) but most of these endemics are rare even where they have been found. The great amplitude of the geographic distribution of the inversions in *D. willistoni* exceeds what is known in such "natural" species as its relative *D. paulistorum*, or as *D. pseudoobscura*, *D. robusta*, and *D. persimilis*, and compares with the situation in "domestic" species like *D. ananassae* and *D. melanogaster*. However, in the latter the wide distribution of inversions is due to transport by man.

Nevertheless, populations of *D. willistoni* are differentiated into geographic races which differ in relative frequencies of certain inversions (table 3). Thus, the samples from Acre contain many inversions, F and E + F in XL, B in II R, and H and L + M in III. Flies from northwestern Amazonia (Rio Branco, Rio Negro) frequently have the combination of the inversions D + H, but not E + F, in XL chromosome, little B in II R, and much L but little L + M in III. The savannas and forests of central and north-central Brazil (Goyaz, Maranhão) have high concentrations of many inversions, among which A, B, D, and D + F + G in XL, B, C, and E in II R, and H in III are conspicuous. São Paulo has much inversion H in XL, D in XR, no A + B in II L, little D, F, H, L, and M, but much J in III. The extreme south of Brazil (Paraná, Rio Grande do Sul) resembles São Paulo, but has less H in XL and much A + B in III. The small samples from Bolivia and from the territory of Guaporé (Porto Velho) seem to resemble Acre, while Marajó is related to Pará, but fails to include some inversions common in the latter territory.

Since the data in table 3 indicate the frequencies of inversion *heterozygotes* they do not reflect the full extent of the racial differentiation of the species. Absence in geographically remote regions of heterozygotes for a certain inversion may be due to the populations of these regions being uniformly homozygous for different gene arrangements. To detect such "racial" gene arrangements, some strains from different regions were crossed to the standard strains from Belem do Pará and from Marajó Island. If the strains crossed differ in gene arrangement, the hybrids will show heterozygous inversions.

Such crosses have indeed shown that populations of *D. willistoni* in different parts of the distribution area have become homozygous for different gene arrangements in some chromosomes. Thus, all hybrids between the standard strain and strains from southern Brazil (São Paulo, Paraná, Rio Grande do Sul) are heterozygous for the inversions C and D, but usually not for B, in XR chromosome. If the standard gene arrangement is denoted bcd, the south Brazilian populations are bCD (although some bCd individuals also occur). In central and northern Brazil all the alternatives of B-b, C-c, and D-d are found. Hybrids of standard with Guatemala (1 strain) and central Mexican strains (4 strains from Axtla, San Luis Potosí, studied) have inversion B but not C or D. Central American strains are, therefore, mostly Bcd. In the XL chromosome, hybrids between standard and south Brazilian strains have the quintuple inversion BDFGH (Plate 1). This indicates that the south Brazilian race is predominantly BDFGH in XL chromosome, while the Pará race, in equatorial Brazil is bdfgh. In central Brazil (Goyaz, Maranhão) the population is mixed. The hybrids of standard and Central American strains are heterozygous for inversions BCD, showing that the Central American race is mostly BCD, while the Brazilian races are all c. In the autosomes, there is relatively little differentiation, although the standard (Pará race) seems to be rather exceptional in predominance of the e gene arrangement

TABLE 4. *Mean numbers of heterozygous inversions per individual* Drosophila willistoni *in different regions of Brazil and in Costa Rica*

Region	Mean	Limits	Region	Mean	Limits
1. Costa Rica	♀3.25±0.85	2–4	12. Monjolinho, Goyaz	♀9.36±0.26	2–14
	♂2.00±0.71	1–3		♂6.56±0.31	2–10
2. Rio Branco, Savanna	♀4.77±0.43	0–11	13. Carolina, Maranhão	♀8.65±0.58	4–13
	♂3.00±0.23	0–7		♂4.33±0.42	1–7
3. Rio Branco, Mucajaí	♀2.67±0.46	0–8	14. Imperatriz, Maranhão	♀5.47±0.37	1–12
	♂2.50±0.49	0–5		♂3.24±0.21	1–6
4. Rio Negro, Amazonas	♀5.03±0.49	1–9	15. Catuní, Bahía	♀0.81±0.03	0–5
	♂4.03±0.36	0–7		♂0.42±0.05	0–2
5. Marajó Island	♀2.05±0.48	0–7	16. Pirassununga São Paulo	♀4.13±0.10	0–11
	♂0.93±0.43	0–5		♂3.52±0.13	0–8
6. Belem, Pará	♀2.85±0.18	0–7	17. Mogi, São Paulo	♀3.97±0.13	0–13
	♂2.53±0.23	0–7		♂2.92±0.34	0–9
7. Western Acre	♀4.68±0.26	0–9	18. Vila Atlantica São Paulo	♀3.06±0.12	0–9
	♂4.58±0.41	0–8		♂2.34±0.12	0–7
8. Eastern Acre	♀5.49±0.31	1–9	19. Paranaguá, Paraná	♀2.61±0.39	0–5
	♂4.86±0.35	1–9		♂2.50±0.68	0–4
9. Guaporé	♀6.39±0.66	1–10	20. Iguassú, Paraná	♀3.20±0.19	0–8
	♂5.25±0.99	3–7		♂2.81±0.22	0–7
10. Santa Cruz, Bolivia	♀5.50±0.73	3–8	21. Reuter, Rio Grande do Sul	♀2.90±0.75	0–7
	♂3.00±1.22	0–7		♂2.40±0.53	1–4
11. Palma, Goyaz	♀9.11±0.44	3–16	22. Santo Angelo, Rio Grande do Sul	♀1.90±0.24	0–4
	♂5.57±0.36	2–9		♂1.25±0.42	0–3

in the II R chromosome, while other regions have chiefly E. Further studies are needed to map the distribution of the chromosomal races in *D. willistoni*.

Geographic Variation in the Frequency of Inversion Heterozygosis

Most interesting and significant proved to be the geographic variations in the average number of heterozygous inversions per individual. The data are summarized in table 4. The observed numbers of heterozygous inversions are higher in females than in males for the simple reason that, having only one X chromosome, a male cannot be heterozygous for any inversion in that chromosome.

Examination of table 4 and the maps in figure 1 and figure 2 shows that the frequencies of heterozygous inversions vary greatly from region to region. In the desert (caatinga) of the state of Bahia a female is heterozygous, on the average, for 0.8 inversions, while in the savannas of central Brazil, in Goyaz, this number is more than ten times as high; a Bahia male carries only some 0.4 heterozygous inversions, compared to about 6 in a Goyaz male. All intermediate values also occur.

The highest frequencies of inversion heterozygosis occur in the central portion of the geographic distribution of the species, in central and northern Brazil—from Goyaz to Acre, and from Maranhão to the territory of Rio Branco, north of the Equator. The frequencies of heterozygosis decline away from this central region, and reach minimal values in the deserts of northeastern Brazil (Bahia), in southern Brazil (Rio Grande do Sul), and in Costa Rica (nothing is known about the situation in Central America, Mexico, and the West Indies). The centripetal gradients are, however, by no means regular: the lowest frequency of inversions is observed in Bahia, which is relatively close to the maximal frequency in Goyaz. A low frequency is found also in Belem, Pará, and Marajó Island; the forested part of the territory of Rio Branco (Mucajaí) has significantly fewer inversions than regions north and south of there (savanna of Rio Branco and Rio Negro). These "irregularities" in the gradients are most significant and, as we hope to show, revealing.

Discussion

Our fundamental assumption is that the chromosomal inversions found in natural populations of *D. willistoni*, either because of position effects or, more likely, because of the genes they carry, influence the adaptive properties of their carriers. This assumption is justified by analogy with the situation in other species of *Drosophila* for which experimental data are available, by the observed temporal changes in some Brazilian populations, and, finally, by some inversion heterozygotes reaching frequencies in natural populations in excess of 50 per cent which would be impossible if the homozygotes and heterozygotes were equally viable. If this assumption is granted, the degree of variability of chromosome structure becomes one of the measures of the adaptive versatility of populations. Now, the data disclose great differences in the amount of chromosomal variability in different bioclimatic regions of Brazil. These differences require explanation.

Superficially considered, the only regularity which appears from the data is that the degree of variability is greatest in populations which reside in the central part of the distribution area of the species, and falls off away from this center. According to Vavilov's (1926) theory of centers of origins of cultivated plant species, the region of the highest diversity of genetic types should be the territory in which the species arose and from which it spread elsewhere. The basin of the Amazon, and particularly that of the Tocantins, would, according to this view, represent the center of origin of *D. willistoni*.

It must be noted, however, that the genetic variability on which Vavilov based

his generalization was, or was supposed to be, adaptively neutral. Vavilov's conception was essentially that the degree of accumulation of neutral genetic variants is more or less proportional to the time during which a numerically large population occupies a territory. This view is not directly applicable to adaptively effective variants, especially to variants acted upon by selective forces as powerful as those affecting inversions in natural populations of *Drosophila*. The distribution of such inversions in space will be governed by natural selection, and not by mutation pressure or by genetic drift. Vavilov's idea must be supplemented.

We submit the working hypothesis that the amount of adaptive polymorphism carried in a population tends to be proportional to the variety of habitats (ecological niches) which this population exploits in a territory it occupies. Now, the evolutionary process which produces adaptive polymorphism, and thereby permits the population to penetrate and to control more and more habitats, requires time. Other things being equal, the longer a territory is occupied by a species the greater will be the supply of adaptive variants, and the greater the variety of ecological niches conquered by the species. As the species expands its geographic distribution, it invades territories in which it is able to occupy progressively fewer habitats. At the margins of the distribution area, unless the species is stopped by an impenetrable geographic barrier, the species has a toehold in only few ecological niches, because it has not had time to evolve adaptive polymorphism that would permit it both to expand its control of the environment locally and to spread further geographically. It is very important in this connection that the gene contents of the same inversion in *D. pseudoobscura* are known to be adaptively different in different parts of the distribution area. This means that an inversion modifies its gene contents in accordance with the adaptive requirements of each geographic region. The fact that many of the inversions of *D. willistoni* occur at least from Costa Rica to southern Brazil certainly does not mean that these inversions are similar in gene content throughout this enormous territory.

The limitation of Vavilov's theory is clearly that the polymorphism, in so far as it is adaptive, is more directly related to the number of ecological niches occupied than to the antiquity of the population itself. It is conceivable that a species arises in an ecologically rather uniform territory, spreads to one containing a large variety of ecological niches, and after some time develops a greater adaptive polymorphism in the latter than in its former home. It is a suggestive fact that the greatest polymorphism in *D. willistoni* is found in the savannas and forests of the basin of the Amazon, a region of one of the richest and most diversified fruit producing floras in the world; *D. willistoni* develops in nature on a great variety of fermenting fruits (Dobzhansky and Pavan, 1950). Geologically, the valley of the Amazon is a new territory, of late Tertiary and Quaternary origin, while central and southern Brazil (Goyaz, São Paulo, Paraná, Bahia and the part of Maranhão along the Tocantins) are more or less ancient. The degree of polymorphism is, thus, not correlated with the geological age of the terrain where a population now lives, but rather with the variety of habitats available there.[2] The information at our disposal does suggest that

[2] It may be interesting to consider from this point of view another generalization, similar in principle to that of Vavilov, set forth even earlier by Matthew (1915). Matthew believed that phyletically advanced types of land animals arise chiefly in the central portion of the Holarctic land mass, while the more primitive forms are pushed out and preserved at continental peripheries and on islands. This may mean that continental centers are biotically more complex than peripheries, and consequently offer more varied challenges to which organisms may respond by adaptive evolutionary changes. The more limited diversity of organic types in peripheral and island biota permits lower rates of evolutionary advance.

the "center of origin" of *D. willistoni* is somewhere in the tropical part of South America, but it does not permit of more precise localization.

Consideration of the variety of ecological niches available to *D. willistoni* in different parts of its distribution area helps to understand the observed variations in the extent of chromosomal polymorphism in the regions studied. Although the southern boundary of the species area is not known, it is unlikely that it extends far southward into Argentina and Uruguay; in the northern Hemisphere *D. willistoni* does not live much outside the tropical zone. The populations of Rio Grande do Sul and Paraná are, thus, geographically marginal, and the low frequency of inversion heterozygotes in them is expected on this basis. Furthermore, *D. willistoni* was a relatively rare species in the two collections made in Rio Grande do Sul, which again suggests that it occupies only a limited variety of ecological niches there. More collecting would, however, be needed to establish this point securely.

Another, and very striking, fact is the low frequency of inversions observed in the interior of Bahia, in northeastern Brazil. This is a region which is climatically close to the limit of toleration for most species of *Drosophila*. Little precipitation falls during most of the year in the "caatinga" deserts, and the short but intense rainy season is quite variable from year to year (Anonymous 1941). The peculiar trees and bushes of this region mostly shed their foliage and become dormant during the severe and prolonged dry season. We have collected mainly in a range of low mountains outside the true caatinga, and yet found there fewer species of *Drosophila* than in any other of the 15 regions of Brazil in which collection was made on a comparable scale. Furthermore, by far the commonest species on the caatinga is *D. nebulosa*, and *D. willistoni* is relatively rare (Dobzhansky and Pavan, 1950). It is clear that the caatinga is ecologically inhospitable to *D. willistoni* which controls only a small array of habitats there, and carries little chromosomal variability. A contrasting situation is found a few hundred kilometers westward, in Goyaz and Maranhão. This is a region of exuberant savanna and gallery forests, in which, despite a pronounced dry season, there is no general dormancy of the vegetation as on the caatinga. A considerable variety of fruits mature throughout the year, and our collecting on them revealed a dense and diversified fauna of species of *Drosophila*, among which *D. willistoni* is the dominant species for at least a part of the year (*D. nebulosa* being the chief competitor). The chromosomal polymorphism reaches its maximum in Goyaz; a female larva heterozygous for 16 inversions, which is the highest number found in any species, was found in the progeny of Goyaz flies (table 4).

Seasonal diversity, vs. relative uniformity of the climate, might be expected to enhance the variety of habitats available for *Drosophila*, and consequently the extent of the chromosomal polymorphism. For a time we believed that our accumulating data on *D. willistoni* supported this hypothesis, only to discover later that the facts fit another view even better, namely, that the amount of polymorphism is greater in abundant and widespread species, and less in ecologically subordinate forms. Indeed, the greatest polymorphism is observed in Goyaz (table 4) which is also characterized by alternation of very arid and rainy seasons. The polymorphism decreases westward (Goyaz—Guaporé—eastern Acre—western Acre), as well as northward (Goyaz—Maranhão—Pará) and southward (Goyaz—Pirassununga—Mogí—Vila Atlantica, table 4), and in every case the seasonal climate variations become less and less pronounced (anonymous, 1941). Western Acre, Pará and Vila Atlantica have relatively equable climates.

The correlation between seasonal climatic changes and chromosomal polymorphism breaks down in Bahia, and es-

pecially on Rio Negro and Rio Branco. Table 4 and figure 1 show that the frequency of inversions is high on Rio Negro, which is the region of one of the most exuberant rainforests in the world and one of the most equable climates in the Western Hemisphere. An only slightly more limited polymorphism is found in the equatorial savanna of Rio Branco (along Rio Uraricoera), where the months from May to July have more than 300 mm. rainfall each, while the months from December to March have less than 50 mm. each. The forested zone of the territory of Rio Branco (along Rio Mucajaí) is intermediate both geographically and climatically between Rio Negro and Uraricoera, and yet it has significantly less chromosomal polymorphism than either of the others. The situation becomes comprehensible if one takes into consideration that *D. willistoni* is a less common species on Mucajaí than its near relative, *D. paulistorum,* while on Rio Negro and in the savannas of Rio Branco *D. willistoni* is the predominant species. The map in figure 2 shows graphically the proportions of *D. willistoni, D. paulistorum, D. tropicalis* and *D. equinoxialis* found in the various collecting regions. These four sibling species are very close morphologically, at least sometimes live together on the same fruits, and may be regarded most immediate ecological competitors (Burla *et al.,* 1949; Dobzhansky and Pavan, 1950).

On Rio Negro about 49 per cent of the *willistoni*-like flies were *D. willistoni* and 43 per cent *D. paulistorum;* in the savanna the percentages are, respectively, 74 and 25; while on Rio Mucajaí *willistoni* is relatively rare, 15 per cent of the total, and *paulistorum* and *equinoxialis* dominant with, respectively, 60 and 25 per cent of the total population. It is clear that, if several related species subdivide among themselves the habitats available in a given region, then each of them will dominate fewer habitats than it might if it were the sole possessor of the environment. And although the population density of a species is not necessarily proportional to the number of ecological niches it controls, by and large the more numerous species may be presumed to be ecologically more versatile than its less abundant competitors. The less abundant species should have, in general, less genetic polymorphism to master the environment than a more abundant one. The relatively low variability of the Mucajaí population is, hence, expected.

A similar situation is observed in the state of São Paulo. At Pirassununga *D. willistoni* is predominant at all seasons (throughout the year, *willistoni* forms 95 per cent, and *paulistorum* 5 per cent of the total), and the population contains relatively many inversions. At Mogí, *willistoni* is dominant in winter but *paulistorum* is dominant in summer, and the inversions are fewer (the difference happens not to be statistically significant for females, but is significant for males, Table 4). At Vila Atlantica, *willistoni* is somewhat less common than *paulistorum* in winter and much less common in summer, and the concentration of inversions is significantly lower than in Mogí.

Most remarkable is the low concentrations of inversions at Belem and on Marajó Island, in Pará. The luxuriant equatorial rainforest near Belem assuredly contains a great variety of adaptive niches for *Drosophila,* and *willistoni*-like species have very dense populations there. However, in these populations, *D. paulistorum* is decidedly more abundant than *D. willistoni,* apparently throughout the year. At Belem, *D. willistoni* dominates only those ecological niches not controlled by the apparently stronger competitor, *D. paulistorum.* The Marajó Island sample came from two ecologically quite different regions: forests near Soure on the eastern coast, and patches of forest near Cape Maguarí. The latter region is a grass-covered plain submerged for several months each year under water, with tree vegetation confined to scattered sand hummocks. The species composition of the *Drosophila* fauna is here unlike any

other place visited, with *willistoni*-like species being a decided minority, and *D. tropicalis* being more common than *D. willistoni*. A small population sample collected in the Maguarí region showed the lowest frequency of inversion heterozygotes (less than 0.5 per female) ever recorded in *D. willistoni*.

The amount of adaptive polymorphism present in the population of a species in a given region is, in general, proportional to the variety of habitats which the species has mastered. The variety of habitats mastered is, however, a function of at least three variables: (1) The number of ecological niches potentially available to a given form of life in a given region. The ecological niches can hardly be enumerated at the present level of knowledge, but it is obvious that a region with a varied fruit-bearing flora can offer more ecological niches for a fruit-feeding insect like *Drosophila* than a region with a limited flora and few fruits. Similarly, predators and parasites may find numerous ecological niches in a region having many species of potential prey or hosts, and water dwelling forms in a region with diversified bodies of water. (2) A species is likely to control more ecological niches in a territory which it inhabits for a long time than in a recently colonized one (Vavilov's "center of origin" factor). (3) Presence of competing species with similar ecological requirements. Related species, if they occur in the same region, subdivide among themselves the available habitats so that each of them is able to exploit fewer habitats than it could have in the absence of competitors. Seasonal constancy or changeability of the climate in a physical sense (temperature, precipitation) appears to be a less important variable in the tropics than it is in temperate countries, although it doubtless influences the number of potentially available habitats. As shown by Dobzhansky and Pavan (1950), the biotic environment of tropical *Drosophila* is seasonally quite variable even in regions with rather uniform climate.

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Summary

Natural populations of *Drosophila willistoni* contain many individuals heterozygous for inverted sections in their chromosomes. At least 40 different inversions have thus far been recorded; inversions occur in every chromosome and chromosome limb; one individual has been found heterozygous for 16 inversions.

Most of the inversions are very widespread geographically. Nevertheless, the incidence of certain gene arrangements is quite different in different parts of the geographic range of the species. Every population can be described in terms of the relative frequencies of the gene arrangements it carries, and different populations are racially distinct.

The relative frequencies of gene ar-

rangements in populations of some localities vary from month to month. Whether these variations are cyclic and seasonal cannot be decided from the available data.

The mean numbers of heterozygous inversions per individual vary in different bioclimatic regions from 0.8 (deserts of Bahia) to 9 (central Goyaz). These variations can be accounted for on the supposition that the amount of chromosomal polymorphism, and in general the amount of adaptive genetic variability, is proportional to the variety of habitats which the species has mastered in the environment of a given region.

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THE IMPORTANCE OF MUTATION RATE GENES IN EVOLUTION

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Introduction

One of the problems of longest interest in genetics and evolution is the spontaneous appearance of gene changes, mutations. It is only within the past thirteen years that it has been clearly shown that the rate of spontaneous mutation depends in part at least upon the genotype of the individual and that certain individual genes are themselves capable of causing marked changes in this rate. (See particularly Demerec, 1927 a, b; Neel, 1942; Mampell, 1943; Ives, 1945.)

The mechanism by which mutagenic agents work—whether they be physical, chemical or genetic agents—is still essentially unknown. It is important, therefore, to report studies on any level showing the kinds and quantities of mutations produced by a given agent under specified conditions. It is particularly interesting to report such studies on a genetic agent derived directly from a natural population. Presumably this type of agent is a major source, possibly *the* major contributor, of new mutations in wild populations. Students of genetics generally recognize that such an agent may play a role of exceptional importance in organic evolution.

The origin of the mutator under consideration and some preliminary studies on it have been presented in Ives (1945). Other studies have appeared in abstract form in Ives (1943, 1947, 1949) and in Ives and Andrews (1946). The major aim of these studies and the present report is to show something of what the gene in question does genetically and to discuss the evolutionary significance of these observations. In two of the earlier studies on mutators the genes were lost within a year or two after their first discovery, for reasons not clearly demonstrated. It seemed more valuable, therefore, to attack the mutagenic properties of the present mutator at once rather than to determine the exact chromosome locus involved. As it turns out, since the gene's mutagenic properties are so complex and since only by its ability to raise the mutation rate is the gene phenotypically recognizable, it will be a task of considerable difficulty to determine even roughly its locus. However, since the gene is now successfully balanced with marked chromosomes, the problem of its exact locus is of minor importance.

This mutator gene has been named *high* (hi). The studies completed so far with *hi* have been particularly designed to show what *hi* could contribute genetically to a given wild population of *Drosophila melanogaster*, as regards gene mutations and chromosome rearrangements, under different controlled genetic and environmental conditions. Specifically, they have measured the rate and variety of second chromosome lethal mutations, of X-chromosome lethal and easily noticeable visible mutations, and of X-chromosome inversions when these are associated with sex-linked lethal or visible mutations. In some of these experiments *hi* was homozygous; in others, heterozygous. In some cases the mutations arose in sperm deposited by young males; in others, in sperm deposited by males aged up to four weeks.

Taken together these studies show the following facts. First, *hi* affects the mutation rate of many genes, but not all of them with equal frequency. It increases the general mutation rate up to ten times the normal rate. Second, whether it acts as a dominant or a recessive appears to

depend on the genotype within which it is acting. Third, in some lines its effect on the mutation rate during spermatogenesis falls off rapidly to, or close to, the normal mutation rate as the males grow old; while in other lines there is no such decrease. Finally, it produces chromosomal rearrangements with measurable frequency.

The Origin of the Mutator

In the summer of 1943 ten lines of wild flies were tested for their second chromosome lethal mutation rate by the use of *Bl/Cy sp*^{2} marker stock. Each of these lines was homozygous for a second chromosome collected in Florida in April and contained their other chromosomes in non-regulated proportions from the Florida wild population and the marker stock. The history of these lines, the method of genetic analysis and a summary of the results obtained were included in Ives (1945).

The detailed data from these tests are presented in table 1. The line number refers to the number of the Florida wild male from which the second chromosome of the line was derived. Chi-square tests of the variance of these samples in various combinations shows the following results, expressed in terms of p, the probability of getting as bad a fit, or worse, to a perfect distribution as a result of random sampling. Considering all ten lines together, p is less than .0001, an extremely unlikely random result. When the highest line, 30, is omitted and the remaining nine lines are tried together, p increases to .14, which is not an unreasonable random result. However, the total chi-square in this case is 12.36 and 9.17 of that total is contributed by line 73. This indicates that line 73 does not fit in well with the other eight lines. When the other eight lines are tried together, p rises to .91, an exceptionally close fit to an even distribution. These eight lines are interpreted as representing one mutation rate of .87 per cent. When lines 30 and 73 are tried together, p is .06, not a good fit. It was also found that line 73 was homozygous for the second chromosome inversion, In(2R)NS, while line 30 carried the normal gene sequence. It is probable, therefore, that 30 and 73 do not carry the same mutator. In any case there can be no doubt that line 30 is clearly a high mutation rate line. All subsequent work has been done with this line, the bearer of *hi*.

Table 1. *Chromosome 2 mutation rate in 10 Florida lines*

Line No.	Tests	Lethals	%
21	247	1	0.40
36	371	4	1.08
41	53	0	0.00
43	215	2	0.93
45	87	0	0.00
54	98	1	1.02
68	255	3	1.18
70	161	2	1.24
Total	1,487	13	0.87
73	235	8	3.40
30	349	25	7.16
All	2,071	46	2.22

Evidence that *high* is in the Second Chromosome

The data already presented suggest strongly that *hi* is a second-chromosome gene, since the second chromosome is the only one by which the ten lines differed in regulated proportions and since there was no significant variation in distribution of the lethals in the families of tests comprising the data of line 30 in table 1. Additional evidence of the same kind came out of testing the effects of *hi* on the sex-linked mutation rate. In the first experiment 7 lethals appeared in 657 tests (Ives, 1945, table 8, item 5), which is as much above the normal rate as the second chromosome rate noted above. A year later, however, only 5 lethals appeared in 1,724 tests of the line 30 stock. The value of p in the comparison of these two samples of data is only .017, a very poor fit, indicating a strong probability that

something had happened to the line 30 stock, possibly a contamination from another wild-phenotype stock in the laboratory.

There remained from the earlier work with line 30 a number of balanced *lethal/Curly* (*le/Cy*) stocks. In each of these lines the lethal chromosome was a descendant of the original line 30 and should also carry *hi*. A cross of two different *le/Cy* lines should then give non-*Cy* flies which were homozygous for *hi*. The second chromosome constitution of these flies should be

$$\frac{le_a\ hi\ +}{+\ hi\ le_b}$$

with subscripts *a* and *b* indicating that the lethals are different loci.

Several such crosses have been made in the course of subsequent experiments. The results indicated the presence of *hi* in the *le* chromosome each time. In a number of instances *le hi/Cy* stocks have grown progressively weaker in viability and fecundity and had to be crossed to other *Cy* stocks for strengthening. In each case the recovered and rejuvenated *le hi/Cy* lines proved to be carriers still of *hi*, as evidenced by a high sex-linked mutation rate. All of the remaining data to be presented in this report have come from such crosses of recovered lines.

In the light of the above evidence there can be little doubt that *hi* is a second chromosome gene. It probably originated in the wild population of Winter Park, Florida, and was transferred from there to the balanced stocks now carrying it. It would require a most extraordinary set of coincidences for it to be in any of the other three chromosomes and still have come through in either balanced or homozygous condition in these stocks.

Methods of Genetic Analysis of the X-Chromosome

Early in the experiments with sex-linked mutation rates it was found that the use of the standard *ClB* technique was impractical because of the presence of a very high proportion of XXY females in our several *ClB* stocks. (Later analysis showed that nearly 50 per cent of the females were of this constitution.) A lucky crossover in our stock of *y ct ras f/ClB* made possible the complete control of this situation. The crossover female was *y ct ras f/ct ras ClB*, hence *ct ras B* in appearance. Later this new chromosome was reduced to *ct ClB* by another crossover. Since this form proved to be more viable and fecund, it was chosen for experimental use.

The essential feature of the new *ClB* stock is the sharing of a common marker gene, *cut* wings, by the two marked chromosomes. Using Oregon-R standard wild stock as a source of normal X-chromosomes, two complementary stocks were established, normal males × *y ct ras f/ct ClB* and *y ct ras f* males × normal/*ct ClB* females. Regular males and *ClB* females are used to continue the stocks. Non-disjunction females—in appearance, *ct B* in the F_1 of the first cross and *B* in the second—can be eliminated at once. They can be similarly eliminated when experimental males are mated to the double-marker females. One could, of course, substitute a third marked chromosome, not carrying *ct*, for the normal one in these stocks and insure himself against the undetected accidental use of non-virgin females in his experimental crosses. In the present case it has proven more advantageous to outcross the marker stock to Oregon-R from time to time to improve the fecundity of the marker stock.

The following method of matings has been used in these tests. Males of the desired constitution with respect to *hi* or its normal allele were mated individually to *y ct ras f/ct ClB* females. The F_1 *ClB* (not *ct*) females, carrying the testing X-chromosomes, were mated to their *y ct ras f* brothers. It was found that if these flies were mated at once in cultures containing a single female and two males each, an annoying amount of sterility, often more than 20 per cent, developed in these test vials. However, if groups of

30 to 35 females were mated with equal numbers of males for two or three days and then put into cultures of one pair each, the sterility was generally less than five per cent. These matings were done in shell vials.

The offspring of the *ClB* test matings were examined under a binocular dissecting microscope without anesthesia. In cases where there were no males, or where the males were obviously abnormal, non-*ClB* females were mated to *y ct ras f* stock males and the locus of the lethal or visible mutation was determined. In a few cases an apparent lethal by *ClB* test proved in larger crossover counts to be either semi-lethal or normal. In such cases, where the chromosome gave a viability of more than five per cent, it was reclassed as non-lethal.

Visible mutants and lethals were grouped together for mutation rate determination. There were approximately eight times as many lethals as visibles, even when including among the visibles some strong semi-lethals which happened to have phenotypic effects. In the tables of data all of these are grouped under lethals.

When it was desired to make more tests of an experimental male than his *ClB* offspring made possible, the sibling non-*ClB* females were also tested in similar manner. Offspring from these tests were normally wild type, *y ct ras f*, or any of the possible crossover types. After some practice I was able to class these tests, too, for lethals and visibles without anesthetizing the flies. The trick seems to be to "photograph" a moving male fly in one's eye and then examine the photograph for the presence or absence of the marker genes. Only *forked* of the four genes used presents difficulty. The most likely loss of lethals through classification error is that group lying several units to the right of *f*. Actually, the proportion of such lethals found in normal/*y ct ras f* tests was as great as in the *ClB* tests. In all doubtful cases the flies were anesthetized for final check. While the classing of normal/*y ct ras f* tests was much slower than the *ClB* tests it was possible to class them much more rapidly without anesthesia. Their use made it possible to secure significant data in many cases that would otherwise have been lost, particularly cases of aged males with greatly reduced fecundity.

Effects of Increasing Age of the Male on the Mutation Rate

In the fall of 1944 experiments were begun to determine the mutation rate manifested in sperm deposited by homozygous *hi* males during the course of their first four weeks of life. Since none of males used in preliminary tests deposited significant numbers of active sperm in the fifth week, these tests cover essentially the lifetime production of *hi* males.

In these experiments the males were raised at 25° C. and mated individually within 24 hours after emergence to two or three marker females. Each male was transferred to a new set of females twice weekly without anesthesia. (The normal male had much better phototropism as well as greater flying ability than the *ct ClB* females.) At the time of his transfer the females were shaken to a new vial to increase their productivity.

Four different *le hi/Cy* stocks were used to produce *hi/hi* males for these experiments. The rest of the 25 stocks did not give sufficiently viable flies in such crosses to be useful. The stocks used were numbers 1, 5, 14 and 29. The crosses proving useful for these tests were 1 ×5, 5 × 14, 5 × 29 and 14 × 29. The most satisfactory of these over several years of time were males of 5/14 and 14/29 constitution. Not all of the combinations were tested at one time, but each one was tested in at least two groups of males.

The experiment was carried on during four two to three months periods in three different years. The results of the different series were consistently alike. They have been pooled for consideration in this report.

The data from analysis of 1/5, 5/29 and 14/29 males indicated that *hi* had similar effects in each set of males. Their pooled totals are presented in table 2A. This table shows the number of completed tests on spermatozoa deposited in each of four consecutive weeks by 27 individually mated males all of whom are represented in each week's tests. These 27 males represented 10 of 1/5, 11 of 5/29 and 6 of 14/29.

A distinction is made between new and duplicate lethals. Whenever a crossover test of two or more lethals from the sperm of one male indicated that they were in apparently the same locus they were considered to represent but a single mutation. They were entered in the column of new lethals as one mutation in the week in which the first one was recovered. The one or more duplicates were entered in the duplicate column in the appropriate week or weeks. Thus the total number of lethal chromosomes recovered is the sum of the new and duplicate columns in each week. The total per cent of recovered lethals is calculated from this combined total. Most of the information concerning activity of *hi*, however, is found in the third column of table 2, per cent of new lethals recovered, since this is directly related to the number of loci which had mutated.

TABLE 2. *Sex-linked mutation rate in sperm deposited during successive weeks after emergence*

A. 27 Males of Combinations 1/5, 5/29 and 14/29

Week	Tests	New le's	%	Dup. le's	Total %
1	1,783	23	1.29	5	1.57
2	1,697	10	0.59	6	0.94
3	1,977	6	0.30	1	0.35
4	1,555	4	0.26	2	0.39
Total	7,012	43	0.61	14	0.81

B. 9 Males of Combination 5/14

Week	Tests	New le's	%	Dup. le's	Total %
1	631	4	0.63	0	0.63
2	642	10	1.56	2	1.87
3	884	11	1.24	2	1.47
4	886	12	1.35	9	2.37
Total	3,043	37	1.22	13	1.64

C. Male No. 32, Combination 5/14

Week	Tests	le Chrom's	%	Corrected B %
1	82	3	3.7	.70
2	122	10	8.2	1.31
3	48	4	8.3	1.18
4	37	2	5.4	1.30
Total	289	19	6.6	1.14

The data of table 2A show that in the combinations represented here the mutation rate falls off rapidly week by week, from a high of 1.29 per cent in the first week to a low of 0.26 per cent in the fourth week, the rate in the third week being essentially the same as that in the fourth. The number of duplicate lethals recovered also decreased sharply in the third and fourth weeks.

Since the sperm of a given male were by no means exhaustively tested the presence of even a few duplicate mutations is enough to indicate that *hi* produced its mutations principally in the spermatagonial cells or their progenitors in the males represented in table 2A. The duplicate lethals represent several loci in different males, apparently at random.

The combination of 5/14 produced quite different results. Data from nine males of this type are shown in table 2B. In this case the homozygous *hi* continued its effect throughout the normal period of spermatogenesis at a rate (note the total rate for the four weeks together) very close to that evident in the first week sperm of males represented in table 2A. If anything, the rate was higher in the last three weeks than in the first week; and the highest proportion of duplicate lethals appeared in the fourth week.

One male, number 32, of combination 5/14, was quite exceptional to the other 36 males tested. The data from this male are presented in table 2C. He produced an exceptionally high frequency of lethal

chromosomes, all of the lethals being in apparently the same locus. From the total of 6.6 per cent lethals it seems probable that this mutation took place early enough so that roughly one-eighth of the spermatagonia of one testis were derived from the mutant cell. It did not seem right to include these duplicates in those of table 2B; but the tests of this male should be added to those of that table for rate of new lethals, these lethals representing one more new mutation recovered in week 1. The last column of table 2C shows the percentage of new lethals in the combined data of tables 2B and 2C. It can be seen that the percentage of new mutations in the total of four weeks, 3,332 tests, is reduced to 1.14, still very close to the 1.29 per cent observed in the first week in the males of table 2A.

No large scale attempt was made to check the mutation rate in non-*hi* males under conditions similar to those obtaining in the experiments recorded above. However, the following stocks were constructed and used in a small scale control test. Line 43 of the stocks recorded in table 1 was chosen as a representative low or normal mutation rate stock. At the beginning this stock was homozygous for its second chromosome and contained not more than four different third chromosomes. Its two lethal sublines, 47 and 60, each *le/Cy*, were crossed in the same way that the *hi* line lethals were crossed. The resultant 47/60 males, homozygous non-*hi*, were then tested in the same manner as the above males for X-chromosome mutation rate, 8 males over a four weeks' period. The tests totaled 928, fairly evenly divided among the four weeks. Two lethals were found, one in sperm of the first week and the other in the fourth week, showing only that the lethal mutation rate is comparatively low in this combination.

Professor H. J. Muller was carrying on experiments with similar purpose at that time in this laboratory (Muller, 1946 and unpublished). It was intended that his results be considered in part a control for the present experiments. However, his methods were somewhat different and he tested X-chromosomes which were not normal, phenotypically and with respect to viability, so that it is not proper to compare the actual mutation rates obtained in the two sets of experiments. His rate in the first week of sperm production was higher than that found in the 47/60 data to be presented later in this report, possibly due to additive effects of new semi-lethal mutations with the lowered viability already present in his test chromosomes.

In Muller's tests of a low mutation rate stock there was a distinct drop in mutation rate in sperm deposited after the first week of activity, a drop which is similar in principle at least to that which appears in table 2A of this report. Zuitin (1941) has reported a similar mutation rate decline with increasing age in his studies on wild-type males derived from each of two natural *Drosophila* populations in Russia. Therefore, the data of table 2B and 2C together are more abnormal than those of table 2A. The males of 5/14 combination are less like normal males than are males of the other crosses.

Dominant Effect of *hi*

Experiments were also planned to test the dominant effect of *hi*. In order to simplify the procedure, tests were made of normal/*hi* which were non-*Cy*. This also made it possible to test the effects of heterozygous chromosomes of recent origin from natural populations.

Control tests of normal (+/+) consisted of earlier data from males of the original Florida 43 stock and from concurrent tests involving males of lethal sublines 47/60. Control tests of *hi/hi* consisted of data gathered from crosses of subline 29 to other subline *le-Cy* stocks derived from the original line 30, principally to subline 14. The *hi*/+ data came from crosses of 29 to 47, 60, 68 and 72. The last two sublines are *le/Cy* stocks isolated from the Florida 43 stock after it had been bred in bottle cultures

for some 70 generations of about 100 pair matings. For one series of tests, lines 29 and 60 were made homozygous for their third chromosomes by means of a *Cy; DcxF* stock, these homozygous lines being noted as 29–1 and 60–4, respectively. Lines 68 and 72 were also homozygous for their third chromosomes. Extensive tests of *hi/hi* were run at approximately six-month intervals to make sure that significant changes did not occur in the *hi* chromosome of line 29.

Because of the effects of ageing on the mutation rate, only sperm deposited in the first ten days were used. Generally, sperm deposited in the first week were enough for the number of tests desired. However, the development of these males was slower in all series than in the previous tests in spite of no obvious change in technique or conditions. Calculated from the time of egg laying, these males were from two to four days older at emergence than were those of earlier experiments. Their sperm in the first ten days may, therefore, have been more nearly equivalent in developmental age to the sperm of the first two weeks in earlier experiments. This may account for the lower mutation rate in *hi/hi* control tests in these experiments on dominant effect of *hi*. Strict care was taken to make sure that *hi/+* males were not different in age from their control males of both types. All of them were raised under similar environmental conditions.

The results of these tests are presented in table 3. The +/+ data are separated into two groups, those coming from tests of the Florida 43 stock itself and those from males of the 47/60 combination. There is obviously no difference between the two rates. Together they show a rate very close to one lethal per thousand tests. At the other extreme are the data from *hi/hi* which show a rate close to one lethal per hundred tests, ten times as high, approximately.

The *hi/hi* data were tested for consistency by dividing the total into eight groups of tests numbering between 969 and 1,758 tests per group, in chronological order. That the variation in mutation rates between groups was random is indicated by the p value of 0.68 for their distribution around their mean. That there

TABLE 3. *Mutation rates in hi/+ crosses*

Cross	Lines	Tests	Lethals	%	Item No.
Control +/+	Fla. ♀	4,618	5	.108	1
	47/60	7,100	7	.099	2
	Total	11,718	12	.102	3
Control *hi/hi*	29/others	10,323	100	.969	4
hi/+	29/75	3,337	7	.210	5
	29/68	4,334	10	.231	6
	Total	7,671	17	.222	7
	29/47	9,206	34	.369	8
	Total	16,877	51	.302	9
	29/60	4,234	33	.779	10
	29–1/60–4	5,917	39	.659	11
	Total	10,151	72	.709	12
hi/+	Grand Total	27,028	123	.455	13

was no gradual shift in rate with time is indicated by the terminal rates of 1.03 per cent in Nov. 1944 and 1.00 in May 1948. The extremes were 0.64 and 1.34 in the two smallest groups, in the spring and fall of 1945.

The results from the *hi*/+ crosses, items 5–13 of table 3, show marked differences between crosses. The results within each cross were, however, consistent. In crosses of 29 by 68 and 75 the mutation rate in *hi*/+ was at most twice the +/+ rate. Neither line is strongly above the control statistically, the p values being .13 for 75 and .050 for 68. Their combined total, item 7, is significantly different from the control with a p value of .038. The mutation rate in 29/47 flies, item 8, is well above the control +/+, the value of p being less than .0001. It is not clearly above the rates of items 5, 6 and 7. When the three crosses are tried together the value of p is .22. When the comparison is between the totals, items 7 and 8, the value of p drops to .080, suggesting that line 47 did produce a slightly higher rate. The difference is not statistically certain, however. The combined total of these three crosses (item 9) is statistically above the control, the value of p being .0004. If one considers these three groups of data (items 5, 6, and 8) as essentially similar, it is clear that they represent a mutation rate above that of the control +/+ tests.

In the tests of *hi*/+ in crosses 29 by 60 and 29–1 by 60–4 (items 10 and 11) the mutation rates are well above the control +/+ rate, the values of p in each case being far below .0001. A p value of .42 indicates that there is no difference between these two rates. A comparison of their total (item 12) with *hi*/*hi* (item 4) gives a p value of .045, a significant though not compelling difference. The other *hi*/+ rates in this table are all statistically far below the *hi*/*hi* rate.

This table of data shows at least two different *hi*/+ mutation rates, one near the +/+ rate and the other near the *hi*/*hi* rate. There is a suggestion that the lower of these two may actually represent two different rates. At most, the data show rates in *hi*/+ that are 2, 4 and 7 times the control rate; at least, they show rates that are 3 and 7 times the normal mutation rate. The rate in *hi*/*hi* is indicated as 10 times the normal rate.

In the data resulting from the tests of *hi*/*hi* and *hi*/+ there were many instances in which one male produced more than one mutant locus in the sample of X-chromosomes tested in his offspring. The occurrence of families of 0, 1, 2 and more lethal loci per family of tested chromosomes should follow a Poisson series if all the males of any one cross produce mutations at the same rate. If some produce at a rate much higher than others, however, the result will not be a Poisson series. Thus, a test for goodness of fit to a Poisson series should indicate if there is marked variability among the males in each cross represented in table 3.

Most of the males tested contributed between 50 and 100 tests to the data. Those few which contributed more than 100 tests were divided into two equal families for the sake of this analysis. Those contributing less than 50 tests were added together consecutively as they appeared in the records to form families of between 50 and 100 tests per group, each group being considered one family of data. When these family limits were strictly followed, it was found that in every cross the number of families with 0, 1, 2 or more lethals was statistically not different from a Poisson series. The fact that most of the values of p lay between .3 and .1 is probably due to the size spread of the families. Actually, family size should be the same, not variable between such limits as used here, when employing a Poisson series test. Otherwise, as in the present instance, one underestimates the value of p. Accordingly, the p values found in the present case may be considered very satisfactory in indicating a Poisson distribution of lethals. This, in turn, indicates that the males within each cross produced mutations at similar rates.

THE EFFECTS OF *hi* ON DIFFERENT LOCI

It is important to know if the effects of *hi* are upon a few genes specifically or if it causes mutations in many different genes. The first experiments shedding light on this question tested the effect of *hi* on the production of second chromosome lethals. It is a simple matter to determine the relationship of such lethals to each other by crosstesting. This was done for a total of 27 lethals which occurred in these tests. The crosstests showed that there had been mutations in 25 loci, two of which had mutated twice each. Data on recurrent lethal mutation rates in normal mutation rate stocks (Ives, 1945) indicated a probability of obtaining in a sample of this size one duplicate lethal due to a second mutation of a locus. It was, therefore, concluded that the effect of *hi* was random in this sample of mutated genes.

Since that first experiment, however, data have come out of the experiments with sex-linked mutations which are not entirely in line with such a simple picture. These data pertain to the occasional visible mutations which occurred in these experiments. Because of the author's own experiences in finding a large proportion of *yellow* alleles among the mutants appearing spontaneously, particularly in the hundreds of *le/Cy* stocks worked with in the past dozen years, it was expected that a large proportion of visibles found in these experiments would be *yellow* alleles. Both Demerec and Neel have reported a high proportion of *yellow* alleles in their studies. As the number of visible mutations accumulated, however, it became obvious that *yellow* alleles were actually rare among the visibles produced by *hi*. Finally, in order to get more accurate data for comparison, records were kept on all sex-linked mutations occurring in about 140 *le/Cy* stocks during the period September, 1948, through June, 1949. As in the *hi* tests, only easily seen visibles were recorded and tested. These included genes affecting eye color, body color, wing size and shape, and bristle size and shape. The stocks contained lethals isolated from the local wild population in the summer of 1948.

The flies in whose germ cells the mutations occurred in the *hi* and *le/Cy* series differed from each other chiefly in their second chromosomes. In the *hi* tests these were both of Florida origin, carried a lethal, and either one or both of them carried *hi*. In the *le/Cy* stocks one second chromosome was *Cy*, with its lethal effect and two inversions; the other was a lethal-bearing chromosome with unknown other factors present, only a small proportion of these chromosomes being closely related to each other. The third and fourth chromosomes were of inbred Florida stock origin in the *hi* series; while in the *le/Cy* stocks, they were on the average three-quarters of this type and one-quarter of local wild population origin. The X-chromosomes, in which the mutations actually took place, were the same in both series and were of inbred Florida stock origin. Inasmuch as *Cy* was not a factor in producing *yellow* alleles in the experiments of Demerec and Neel, the major contrast in these two series is between the effects of *hi* on the one hand and of a random sample of second chromosome wild-population dominant genes on the other. These latter represent an estimate of what could be expected to happen in the highly heterozygous wild population from which they came and are accordingly called controls. An estimated 70,000 males were examined in these control tests.

The data from these two series of tests are summarized in table 4. The most striking difference between the two series is the presence of many *yellow* alleles in the control group, each one of which occurred in a different *le/Cy* stock. Only one of 42 visible mutations in the *hi* tests was a *yellow* allele; 19 of 37 mutations noted in the *le/Cy* stocks were *yellow* alleles. The sterile mutants in the control group are included because of the

strong bilateral symmetry of the phenotypes. Most of these six cases also occurred in more than one male in their respective stocks. Some of the *hi* visibles were known to be male-sterile; but not all of them were tested for this characteristic, since the necessary data on locus and allelism could be obtained from heterozygous females. With the exception of *yellow*, no locus was found certainly more than twice in the controls. One of the steriles may have been a third occurrence of *forked*. It looked more like *singed*, but there is no absolute difference between these two phenotypes. Of the three mutants occurring twice in the controls, two —*lz* and *w*—occurred once in the *hi* series. Found once in each series was *wy*.

The data on *folded* (*fo*) wing are of special interest. None was found in the controls. More than that, I have tested seemingly dozens of *folded*-like flies in the past 18 years without once finding an allele of folded—until these in the *hi* series appeared. It does not seem to be a locus with a mutation rate comparable to that of *y*, *w*, *pn*, *sn*, *lz*, *ras*, *m*, *v*, *wy*, *g* or *f*, each of which has appeared several times in this laboratory. Yet, here *fo* appeared four times (in unrelated sperm) in tests of *hi*/+.

The general conclusion from this table of data is that certainly the frequency of mutations at the *yellow* locus, and very probably that at the *folded* locus, are different in the two series of tests. The mutation rate of the *y* locus seems to be either not affected at all, or else actually decelerated, by *hi;* while that of the *fo* locus seems to be accelerated more than the others.

A comparison of the data on sex-linked visibles and second chromosome lethals in tests of *hi* shows that in both instances many different genes were affected. No such case as *y* could be expected to appear in the second chromosome lethal tests; and the data on *fo* stand out because of the complete lack of *fo* alleles in previous mutation work, not because of any statistical difference (which is nil) between its rate of mutation in this experiment and the mutation rate of the two recurrent second chromosome lethals. Thus it can be seen that the data on sex-linked visibles are an extension of the findings with second chromosome lethals. The added information of specific effect on the *y* and *fo* loci results from advantages associated with the use of data on sex-linked visible mutations.

TABLE 4. *Kinds of sex-linked visible mutations*

Class	No. Mut's.	Steriles	y's	Loci> 2×	Loci 2×	Loci once
Control	37	6	19	none	*f*, *lz*, *w*	6
high	42	?	1	*fo*(4)	none	37

THE RATE OF X-CHROMOSOME INVERSIONS

Because lethal and visible mutations in the X-chromosome were tested to determine their locations on the chromosome, it was possible to determine at once if one of these mutations was associated with an inversion. A total of 351 mutant chromosomes so tested revealed 17 cases in which crossingover was so reduced in repeated tests in one or more regions that an inversion was indicated. In addition, two other probable inversion chromosomes were lost before retests could be made. Of the 17 inversions that were certain, 16 were associated with lethals and one was associated with a visible mutation of good viability. The associated lethals and visible were not tested further but are presumably position effects of the inversions. The ratio of non-inversion to inversion chromosomes in these 351 tests is roughly 19 to 1.

Crossover data are not sufficient to indicate more than the approximate location of an inversion in the chromosome. This requires the study of salivary gland chromosome preparations. *Dr. Taylor Hinton* of this laboratory has begun such an analysis of those of the above inversions, 12 in number, which have been successfully continued in stock cultures.

Preliminary analysis indicates that the distribution of breaks in the chromosome is probably similar to that reported by Kaufmann (1946) in a large sample of breaks induced by X-rays.

Discussion

1. The effects of ageing

The data presented in table 2 show that the effects of the mutation rate gene, *hi*, depend upon additional genetic factors. In this instance, crosses of four *le hi/Cy* stocks to each other to produce *hi/hi* flies revealed one combination, the cross of line 5 to line 14, which gave distinctly different results than came from the other three crosses. The design of the experiments was not such as to reveal precisely the genetics of this difference; but the consistency of the results leaves little doubt that the difference was genetic and that the factors responsible were located in the *le hi* second chromosomes. There are several possible genetic means by which the observed results could have been achieved. The simplest hypothesis is that before the extraction of these four sublines from the original stock a recessive mutation occurred and was eventually included in those chromosomes leading to lines 5 and 14, but not in those chromosomes which were eventually isolated in lines 1 and 29. This recessive gene, homozygous in 5/14 flies, then modified the effect of *hi* in the manner indicated by the data.

While the direct genetic cause of the differences observed is obscure, it is possible to analyze the result of this cause and to suggest how it may have been produced. The mutation rate in the three crosses which were alike (table 2A) was characterized by being high in sperm deposited in the first week and dropping off rapidly in sperm deposited in subsequent weeks. This phenomenon was first observed in the X-chromosome lethal mutation rate following X-ray treatment and has been explained in part by germinal selection (Timofeef-Ressovsky, 1937). In the present instance, the number of duplicate lethals also decreased sharply after the second week, as would be expected if germinal selection were the cause of the drop in the observed mutation rate. Apparently, in this case, spermatogonial cells carrying a lethal-bearing X-chromosome generally cease producing spermatocytes (perhaps because of metabolic deficiencies resulting from the lethal gene) before normal spermatogonial cells do. In addition, it appears that *hi* produces most of its effect when the males are young. Possibly it ceases to function after the males have emerged as adults. These conditions would cause a sharp drop in mutation rate such as observed in these crosses.

In the case of 5/14 males, the mutation rate did not drop as the males grew older and the proportion of duplicate lethals clearly increased. The effect of the postulated modifying gene seems to be to extend the active period of *hi* so that it causes the production of new lethal-bearing spermatogonial cells at least as fast as the old ones are inactivated by germinal selection. The active period of *hi* in the spermatogonial tissue apparently continues through the period of spermatocyte formation in the 5/14 males.

Obviously, the modification present in the 5/14 cross is one of evolutionary importance. Males of this type produce twice as many mutations in their lifetime as do males not homozygous for this modified condition. Old males of this type produce four or five times as many mutations as occur in old males of the unmodified type.

An attempt was made to test the postulates involved in this analysis by experiments with *hi/hi* females from the same crosses. Since there is no germinal selection of recessive lethals in females, the mutation rate would not drop in any of the crosses and it should increase in the 5/14 cross. The females produced in these crosses were consistently low in viability and productivity and could not be used for such tests.

The case of male number 32 (table 2C)

is of interest because it represents an instance in which spermatogonial cells carrying a certain lethal gene hemizygously were unaffected by germinal selection. Apparently the normal allele of this lethal gene is not involved in spermatogenesis. Or, possibly the lethal allele is not deleterious in its effect on the spermatogonia during spermatogenesis. There was no other case of this kind in these experiments.

2. The significance of variable dominant effects

The data of table 3 show that the mutation rate in *hi*/+ flies also depends upon the genotype in which *hi* is working. The consistency of the results obtained would be expected only if the additional genetic factors were brought in by the + second chromosome in each case. The + chromosome of each of the two lowest rate *hi*/+ crosses was derived from the original + stock many generations after the extraction of the two higher rate + chromosomes. They could have carried a mutant modifying gene not found in the earlier ones. Presumably this is a dominant modifying gene. Since it traces back to a different stock than the modifying gene postulated in the analysis of ageing effects, it need not be an allele of that gene.

The relationship of *hi* and the two postulated modifying genes to each other is not indicated by any of the experiments of this report. One can reasonably predict from these results, however, even without specific knowledge of the genetic nature of these particular modifying agents, that the effectiveness of mutators in a wild population depends very much upon the genetic modifying agents which are present or which arise in the population.

A mutator which can act sometimes as a dominant and sometimes as a recessive should produce a longer and more lasting effect in a natural population than one which is either completely dominant or recessive. The completely dominant gene will be quickly eliminated along with the bad mutations it produces. The completely recessive mutator will produce very little effect when present in only a small portion of the chromosomes of the population, since even in comparatively small natural populations of *Drosophila* the actual amount of inbreeding and homozygosis is low (Dobzhansky, 1941).

In the present case, *hi* would probably contribute most of its mutations while acting as a dominant gene and would be continued from one generation to the next, without significant loss due to selection, in flies in which it acted as a recessive. Since it was derived from an essentially large population, the probability of its complete elimination by chance (without selection) is at a minimum. It should continue in existence much longer than it would in a small population where chance elimination of genes existing in low frequency is often greater than elimination by selection.

3. The mutation rate in large vs. small populations

Because mutators like *hi* have a longer span of existence, on the average, in a large population than in a small one, large populations should have, generally, the higher frequency of active mutators. Accordingly, the mutation rate in large populations should be higher than in small populations, on the average, the difference in rate depending both upon the rate of appearance of new mutators and the effective difference in population size. Since laboratory stocks breed in small sized populations in bottle or vial cultures, such stocks should normally have mutation rates well below those present in natural populations in the case of *D. melanogaster*. The reduction of heterozygosis by chance, together with natural selection against mutators producing high mutation rates, should eliminate such mutator alleles comparatively rapidly in laboratory stocks. Laboratory stocks only recently derived from a natural population should carry more active mutators (and their modifiers) than stocks of long lab-

oratory history and should therefore show higher mutation rates.

Zuitin (1941) has reported just such a difference in spontaneous mutation rate between natural populations and a laboratory stock of long standing. In his case, there was an X-chromosome lethal mutation rate of 0.62 per cent in 7,776 tests of males whose chromosomes were entirely of recent Russian natural population origin, in contrast to a rate of 0.16 per cent in 8,614 tests of males from a standard laboratory stock of American origin. The data of Olenov *et al.* (1939) and of Zuitin and Pavlovetz (1940) show spontaneous mutation rates in both sex-linked and autosomal genes which are generally above those found in normal laboratory stocks when they tested flies whose chromosomes were either wholly or partly of wild population origin. The quoted data of Zuitin above are particularly indicative that the act of hybridizing laboratory and natural population chromosomes is not the major cause of increased mutation rate (by genic interaction). They show that genes causing a higher level of mutation rate are already present in the wild population chromosomes.

None of the many stocks of long standing in our laboratory has ever shown a mutation rate for sex-linked visibles comparable to that observed consistently through the past twelve years in collections of *le/Cy* stocks in the year following their isolation from different wild populations. The data of the present study can also be used to demonstrate this point. The mutation rate in the control +/+ flies of table 3 is representative of that in a stock of long laboratory history. The sample of *le/Cy* stocks under observation through the 1948–1949 period represents stocks recently isolated from the wild population. By using the 8:1 ratio of lethals to visibles which was observed in the experiments with *hi*, one can calculate that there was a total mutation rate in these stocks which was not less than four times the control rate. This is a minimum estimate, because many of the semilethal visibles on which the 8:1 ratio is based would fail to reach maturity and would not be observed, when occurring in the *le/Cy* stocks. It seems most probable that dominant mutators, brought in principally in the second chromosomes from the wild population, were the major cause of the significant increase in mutation rate in the *le/Cy* stocks.

The total frequency of mutators can hardly be as great in the *le/Cy* stocks as it is in the natural population from which they came, since most of the third chromosomes of these stocks are of inbred laboratory stock origin and would therefore carry alleles causing only low mutation rates. The natural population is known to carry a large amount of genetic variability in all of its major autosomes. The mutation rate in the natural population, therefore, should be higher than that observed in the *le/Cy* stocks.

The above data and considerations do not support the conclusions of Sturtevant (1937), whose theoretical analysis led him to infer that mutation rates determined by the use of highly inbred stocks and the usual mating procedures are probably higher than those which occur in nature. The reverse seems more likely to be true.

4. The significance of differential effects on various loci

The differential mutation rates of *folded* and *yellow* in the present experiments suggest that mutation rate genes can endow a natural population with a potentiality for a kind of directed evolution. A population carrying *hi* in ten per cent or more of its chromosomes would be offered a much larger proportion of alleles of *folded* than would occur in a population lacking *hi*. It would receive fewer alleles of *yellow* than would populations carrying either the Demerec or the Neel mutator. Thus, if alleles of *folded* or *yellow* became of selective value, such populations would diverge from each other much more rapidly than a knowledge of the simple average mutation rate

of all genes combined might lead one to suspect.

The cases of *folded* and *yellow* are used here only as potential examples. They are mutations which are at present selectively bad. There are, however, paleontological instances (Wood, 1947, for example) in which evolutionary rise of species has occurred much more rapidly than normal mutation rates seem to allow, apparently as a result of a series of rapid adaptive changes in individual organs or systems. High mutation rate genes, accelerating the production of mutations causing such specific changes, could be an important contributory means of explaining such paleontological findings. Because of their specific as well as their general effects on mutation rates, then, mutators may be factors of prime importance in evolution.

5. The significance of the production of inversions

The data of this report indicate that *hi* produces chromosomal inversions at a rate of approximately one inversion to 19 lethal or marked visible mutations. The inversions occurred in both homozygous and heterozygous *hi* flies. Presumably, inversions occur in each of the four arms of the major autosomes at a rate approximately equal to that found for the X-chromosome. This leads to an estimated minimum rate of inversions amounting to approximately one in 400 germ cells for *hi/hi* and to between one and four per 2,000 germ cells in *hi/+* flies. The actual rates may be well above these estimates since they will include inversions not associated with position effect lethals and visibles. Also, the actual lethal mutation rate of *hi/hi* in the two arms of the second chromosome was seven to eight times that in the X-chromosome, not just twice as high.

Inversions and translocations have occurred so rarely spontaneously in tests of average laboratory stocks that it has seemed possible that those which do occur may result from natural radiation. (See Plough, 1941, and the discussion following that paper.) The much higher inversion rate in the presence of *hi*, without increased radiation, indicates that radiation is not necessary to account for spontaneous inversions any more than it is to account for spontaneous mutations in general.

The now large amount of literature on inversions in natural populations of Drosophila is ample to show the importance of these changes in gene arrangement both in maintaining the population (Dobzhansky and Levene, 1948) and in supporting incipient evolutionary changes (Sturtevant and Novitski, 1941; Ives, 1947; Dobzhansky, 1948 and others). It is understood, of course, that generally it is not the inversions themselves but the genes included in them that have such effects.

Any agent capable of producing inversions at an appreciable rate in natural populations becomes at once an important factor in evolution. Mutator genes can, therefore, be considered important factors because of the inversions they produce, as well as for their gene mutations, in natural populations.

6. The nature of hi and its effects

At first glance it may appear that *hi* is the same gene which was reported by Demerec (1937). Both genes are in the second chromosome and originated in Florida stocks. There are three considerations, however, which make this relationship seem unlikely: the actual origin of the genes, the mutation rates observed, and the visible mutations found in experiments with the mutators. The Demerec mutator appeared in a Florida stock which had been bred in comparatively small cultures for many years before Demerec worked with it. The present mutator came directly from the wild population in Winter Park, Florida, to the balanced stocks now carrying it. Under these circumstances, it is unlikely that the two mutators are descended from the same mutation. The Demerec mutator showed a

mutation rate (not including duplicates) which was approximately one-third as large as the rate reported here. This could be due to possible heterozygosis of the Demerec mutator in some of his tests and may not be a true difference. Most critical of all is the fact that the Demerec gene produced an abundance of *yellow* mutants while *hi* produces virtually no *yellows*.[1] This is a clear-cut difference. The two genes may be alleles; there is no evidence either way on that point.

Since Neel's (1942) mutation was located on the third chromosome and Mampell's (1943) is in a different species and has different characteristics, it can be stated at once that *hi* is genetically different from each of those genes.

The nature of the genetic effect of *hi* is largely obscure. The time of its effect has already been discussed. Its effect seems to be limited to germinal tissue, since no increases have been noted in the frequency of unilateral somatic mutations in the experiments with *hi*. If *hi* produced this type of anomaly up to ten times as often as it normally occurs, the results could hardly have gone unnoticed, particularly since the author was aware that such events might occur more frequently in stocks and tests of *hi*. It seems very unlikely that *hi* is as effective in somatic as in germinal tissue.

How *hi* produces its effects can, at present, only be conjectured. Generally accepted today is the view that every gene achieves its phenotypical ends by biochemical means, perhaps by the production of a specific enzyme. On this view, *hi* might produce an enzyme which competes with the enzymes involved in the normal reproductive synthesis of genes. When it is successful in this competition, the ultimate result is the synthesis of a mutant instead of a normal gene. The effect of such a common environmental agent as temperature on the mutation rate (Muller, 1928; Plough and Ives, 1935; Plough, 1942 and others) may result from its effects on the activity of mutator alleles or their products, although the earlier studies on *hi* (Ives, 1945) failed to show temperature effects in this particular case. No experiments have been attempted to indicate the nature of an enzymatic activity of *hi*.

7. *General conclusions on the importance of mutation rate genes*

In the foregoing discussion emphasis has been placed upon the probable importance of mutation rate genes as a source of new genetic variability in natural populations. A question which arises naturally from this discussion is whether there are actually any spontaneous mutations. Natural radiation is undoubtedly the cause of some mutations, but it is not generally believed to be normally an important source (Muller and Mott-Smith, 1930). Temperature effects, as pointed out above, may be expressed possibly only through their effects upon mutation rate genes or their products. It is quite conceivable that spontaneous mutations in general result from the changes produced by mutators in the chemical environment in which genes reproduce themselves. On this view, the difference between a low mutation rate stock and a high one is not the absence and presence, respectively, of a high mutation rate gene. It is, instead, the difference in the alleles of mutators which are present in the two stocks. In the case of the low stock, the mutator alleles present are ones whose products cause only a low rate of change in other genes during their reproduction.

[1] It has been suggested that this may reflect a difference in the normal alleles of *yellow* used in the two studies. The normal alleles were not closely related but both have shown themselves to be potentially highly mutable. In the present case the normal allele showed a relative mutation rate (control of table 4) similar to the relative mutation rate observed by Demerec. From this it appears that the two normal alleles were essentially similar in this important characteristic and that the Demerec allele was not a less stable one than the allele used in the present study.

On this view, by far the majority of mutations occurring in a natural population arise from causes *within the cells* of the individual members of the population. Spontaneous mutation is to be thought of not as a "chance" failure of a gene to reproduce itself exactly, but as the result of the influence of the biochemical product of one gene upon the reproductive synthesis of another.

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Summary

Experiments with a high mutation rate gene derived from a natural population of *Drosophila melanogaster* show that this second-chromosome gene produces mutations in many other genes at an average rate of ten times the normal rate when acting homozygously and two to seven times normal when acting heterozygously. Genetic modifiers affect both its dominance and the duration of its activity in the production of sex-linked mutations. Its major effect is in germinal tissue where it produces both lethal and visible mutations at a ratio of approximately 8 : 1. Inversions are also produced. An average of one mutation in twenty was associated with an inversion. The calculated minimum rate of inversion in all chromosomes is one per 400 germ cells in homozygous *high* flies and one to four per 2,000 germ cells in heterozygous flies. The mutation rate of the gene *folded* is increased more than that of other genes, while that of *yellow* is not increased by *high*.

The mutation rate in stocks recently isolated from the local wild population was calculated to be at least four times as high as that in an inbred laboratory stock. Theoretical considerations lead to the interpretation that this reflects the presence of many high mutation rate genes in natural populations.

The evolutionary importance of mutator genes, as exemplified by the present data, is discussed. The thesis is advanced that mutators are the major cause of both gene mutations and inversions in natural populations, and that the majority of "spontaneous" mutations are caused by the biochemical activity of mutation rate genes.

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ECOLOGICAL FACTORS IN SPECIATION OF *PEROMYSCUS*

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Introduction

Mice of the American genus *Peromyscus* are among the several kinds of animals that are particularly useful for study of the factors involved in speciation. This genus is widely distributed over North and Central America, and one or more of the fifty-five named species occurs in almost every part of these areas. Representatives of the genus occupy a wide variety of environments, and a wide range of ecological preferences is found even within a single, widely distributed species.

This genus comprises sixteen or more species groups, or groups of species with close phylogenetic relationships. The species groups of *Peromyscus,* as arranged on morphological resemblance by Osgood (1909) are important evolutionary categories. Members of the same species group, tested in the laboratory, are at least partially interfertile, while members of different groups fail to cross (Dice, 1933a). The species groups of *Peromyscus* have been called cenospecies by Blair (1943a) following Turesson (1922) because of their apparent inability to interchange genetic materials. The species within the groups that have been studied show a wide range of difference in the degree of differentiation. The species within a group, in other words, represent varying stages in speciation, which permits reconstruction of the possible course of evolution of a new species.

Other advantages of *Peromyscus* for evolutionary studies are numerous. Osgood's (1909) revision of the genus, based on more than 27,000 specimens, is outstanding among mammalian monographs that have used the methods of museum taxonomy. Most species of *Peromyscus* are readily adaptable to laboratory life, and consequently to breeding experiment. This fact was first recognized by Sumner (1932, summary), who pioneered in hybridization experiments and in the analysis of intraspecific geographic variation. It was exploited by Dice (numerous papers) principally in analyzing geographic variation in several wide-ranging species. An additional advantage of *Peromyscus* for evolutionary research results from the comparative ease with which the ecological relations of these mice may be determined. The ecological distribution and the habitat preferences of several species are fairly well known. Population densities and social behavior in natural populations of several species have been determined in recent years, following the lead of Burt (1940) and Blair (1940).

Major disadvantages of *Peromyscus* for evolutionary studies are: (1) cytological investigations are not profitable because of the large number (forty-eight to fifty-two) and the small size of the chromosomes (see Cross, 1931), (2) the genetic bases of most wild phenotypes are difficult and laborious to analyze because of the presumably large number of genes with minor additive effects that are usually involved in coat color and in size of body parts. These difficulties, of course, are not limited to *Peromyscus.*

My purpose here is to summarize what is presently known about geographic differentiation and speciation in *Peromyscus* and to indicate some major evolutionary problems to which work in this group may offer an eventual solution. Excluding museum studies, the history of *Peromyscus* as a medium for the study of evolutionary processes traces back some thirty-seven years to the beginning of Sumner's work. Most of the work with variation and speciation in this genus

has been done within the last twenty years due to the efforts of Dice and his associates and students. This work has clarified the relationship between geographic variation and environmental factors, and it has outlined the possible course of evolution of separate-breeding, species populations. The possibilities of this group for evolutionary studies have been barely touched upon, however, and some fundamental problems of speciation are probably more susceptible to attack here than in other animal groups.

The concepts of taxonomic groups that have resulted from modern studies of *Peromyscus* are stated by Dice (1940a, 1940b) and by Blair (1943a). Subspecies are adaptive modifications of parts of the species population. The terms subspecies, geographic race, and ecological race are synonymous. Species are populations that are reproductively isolated (more or less completely) from other, related populations. Our concept of the species is essentially the same as that developed by modern workers with speciation in other groups and expressed by Mayr (1949).

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Causes of Geographic Variation

Geographic variation in morphological characters is evident in nearly all species of *Peromyscus* that have been studied. Samples from populations only a few miles apart may show significant differences in body dimensions and in pelage color (Sumner, 1926, 1932; Dice, 1937a). Within the range of a single species, geographic races may occur that are remarkably dissimilar in body proportions and pelage color but that are connected by intergrading populations. This geographic variation, whether of lesser or greater magnitude, presumably results from the interplay of: (1) mutations, (2) selection by the environment and (3) reduced gene flow through partial isolation of large or small segments of the species population. Mutations provide the materials for geographic variation. Selection by the local environment for or against these mutations results in a local population that, on the average, is best adapted to local conditions. Semi-isolation of local colonies retards gene flow between colonies and may either augment the effects of selection, or it may allow non-adaptive differentiation (Wright, 1943). Modern evolutionary theory takes cognizance of all of these factors in differentiation. The modern concept of the interrelationships of these factors has been restated recently by Simpson (1949, Chap. 14). We will confine ourselves here to discussion of the probable role of each of these major factors in the geographic differentiation of *Peromyscus*.

Mutation

Virtually nothing is known about mutation rates in *Peromyscus*. Present knowledge of the mode of inheritance of the morphological characters that distinguish subspecies and species is limited, and it is here that much work is needed. Various mutations affecting pelage color, coat pattern, or behavior have been isolated and described from laboratory stocks (Sumner, 1932; Clark, 1938; Watson, 1939; and others). These appear, for the most part, to be deleterious mutations, and, while they appear occasionally in wild populations, they are not the characters on which geographic races or species are differentiated. Wild phenotypes appear, in most cases, to be produced by many genes, with several genes having minor additive effects on the same character. This makes genetic analysis very difficult. Some exceptions do occur, and these should permit the measurement of geographic variation at the level of gene frequencies. Allelic genes having major effects on pelage color were described in *Peromyscus maniculatus blandus* by Dice (1933b). Buff and gray pelage color are inherited in simple mendelian fashion, with buff dominant over gray. Dichromatism apparently resulting from the action of

this gene pair was first described from four different subspecies of *Peromyscus maniculatus* in the southwestern United States by Osgood (1909). Examination of field-caught specimens and cross matings in the laboratory show that the buff allele predominates in most geographic races of *maniculatus,* and that the gray occurs in several widely separated localities (Blair, 1947a). Another pair of alleles (black-tipped, brown-tipped) affecting intensity of pelage color has been described from *Peromyscus maniculatus blandus* by Blair (1947b). In *Peromyscus polionotus,* a pair of alleles (white cheek, colored cheek) with major effects on the distribution of pigmented hairs on the body and a complex of other genes with minor additive effects on pigment distribution account for the major morphological differences between geographic races of this species (Sumner, 1926, 1930; Blair, 1944). A group of striking mutations in pelage color and pattern appears to have become established in populations of *maniculatus* occupying coastal islands off the coast of British Columbia (McCabe and Cowan, 1945). No analysis of the heredity of these pattern and color types has yet been published.

Selection

Selection of adaptive genotypes by the environment seems to be a major factor in geographic differentiation of *Peromyscus,* although direct experimental evidence is scanty. Any attribute, whether morphological, physiological, psychological or social, is potentially subject to selection. Selection is, therefore, a complicated process. Most of the evidence from *Peromyscus* concerns possible selection by predators. These mice, with various other rodents, supply most of the food for several species of predatory birds, mammals and reptiles. This is well known from food-habits studies too numerous to list here. What is less well known is the predation pressure on any given population, but there is some information on this point.

The average life span of *Peromyscus maniculatus bairdii* and *Peromyscus leucopus noveboracensis* in nature in southern Michigan was studied by live-trapping and marking the resident mice over a five-year period (Blair, 1948). Among mice that had survived to the dispersal phase in the life history, the average life span was found to be less than five months, although the potential life span in these species is in excess of three or more years. A comparable population turnover was found in *Peromyscus polionotus leucocephalus* on Santa Rosa Island, Florida (Blair, 1950). In both of these cases, predation pressure appeared to be the major factor responsible for the short life span and rapid turnover in the *Peromyscus* populations. There are other causes of death than predation, of course, in natural populations, and these may sometimes have major effects. Howard (1949) attributed higher spring than fall mortality rates in *Peromyscus maniculatus bairdii* to unfavorable weather and starvation in spring. He attributed mortality in his populations to predation and to cold weather accompanied by a shortage of food. The mere fact of heavy predation pressure does not prove that natural selection is operative, for there is no indication whether or not the predators discriminate between adaptive and nonadaptive phenotypes. It does indicate, however, that the mechanism for intensive selection does exist.

The only experiment designed to test the ability of normal predators on *Peromyscus* to discriminate between concealing and non-concealing colors of pelage was performed by Dice (1947). Barn owls (*Tyto alba pratincola*) and long-eared owls (*Asio wilsonianus*), under simulated night-time illuminations, took significantly more mice that were conspicuous on their sand background than they did of mice that resembled the background. A lattice-work "jungle" was used to simulate natural conditions and to increase the difficulty of the owls in catching the mice. Dice (1949) com-

puted a "selection index" and found that selection indexes from his experiments with predation of owls on *Peromyscus* were mostly of the same order of magnitude as was obtained by Sumner (1935a, 1935b) in experiments with predation on fishes. This work should be continued. We would like to know the specific predators that control selection on given species of *Peromyscus*. We would like to know how great a difference has to be in order to be selected, and we would like to know the rate of selection for characters of varying degrees of apparent adaptive value.

The dearth of direct, experimental evidence for selection is remarkable in view of the important role of this phenomenon in modern theories of evolution. Measurement of the rate of selection in the laboratory is feasible, as the experiments of Sumner and of Dice have shown. Field experiments to measure the rate of evolution in adaptive characters under semi-natural conditions also should be undertaken with *Peromyscus*.

Our discussion of selection so far has concerned itself principally with protective coloration in relation to predation. Other morphological variations, as in body dimensions, may be adaptive to the environment occupied, and they may or may not be related to predator selection. In general, semi-arboreal, forest-inhabiting forms have long tails and large feet in *Peromyscus*, while semi-fossorial, grassland forms have short tails and small feet. We have emphasized selection in its rawest sense, i.e., the elimination of the least adapted by predation. This does not mean that we overlook the fact that selection may occur in many ways, often without involving the idea of escape, or concealment, or protection of the individual. Any attribute that may adapt or disadapt the animal to its biological and physical environment is subject to selection by that environment. Elimination of the least adapted does, however, appear to be a major selective agency. A form that is the "bottom" animal in the food cycle and that has a life expectancy in nature of one-tenth or less of its physiological life span must have a life history that involves something of the Darwinian "struggle for existence." Simpson (1949, p. 224) defines natural selection as, "a process of differential reproduction." In a form such as *Peromyscus*, differential reproduction apparently results chiefly from differential survival under conditions of strong predation pressure on the population, although it is certainly influenced by other factors.

Isolation

A third factor in the geographic variation of *Peromyscus* is semi-isolation of a part, or parts, of the species population. Partial isolation of local populations results in reduced interbreeding, and consequently in reduced gene flow, between these populations. The effect of this reduction of gene flow between sub-populations might be to accelerate adaptive differentiation, or it might result in purely random differentiation in non-adaptive characters (Wright, 1943).

The pattern of distribution of the species, an ecological and biogeographical phenomenon, is the starting point for analyzing the role of isolation in intraspecific, geographic variation. Two important facts relating to isolation within the species population stand out from ecological field studies of *Peromyscus*: (1) the individual is localized to a few acres or less of terrain by its home-range habit; (2) the species is always unevenly distributed within the geographic limits of its range.

A mouse, upon reaching sexual maturity, establishes itself in a given area, and future movements of that individual are largely confined to an area of a few acres. Home-range size in *Peromyscus maniculatus* has been estimated by live-trapping and marking methods in several different environments (table 1). Even where the mice ranged most widely, in the mesquite association of southern New Mexico, the average home range com-

TABLE 1. *Estimated size of home range in three species of* Peromyscus, *from live-trapping and marking records*

Species	Locality	Ecological association	Home range in acres	Source of data
P. maniculatus bairdii	Michigan	Bluegrass	5–6	Howard (1949)
P. maniculatus gracilis	Michigan	Beech-maple	♀ 1.39± .16 ♂ 2.31± .27	Blair (1942)
P. maniculatus blandus	New Mexico	Mesquite	♀ 4.10± .39 ♂ 4.66± .33	Blair (1943b)
P. polionotus leucocephalus	Florida	Dense grass	4.76± .74	Blair (1950)
P. polionotus leucocephalus	Florida	Open grass	10.66±1.46	Blair (1950)
P. leucopus noveboracensis	Michigan	Oak-hickory	♀ 0.21 ♂ 0.27	Burt (1940)
P. leucopus tornillo	New Mexico	Mesquite	♀ 4.5 ♂ 3.1	Blair (1493b)

prised less than five acres. Home ranges of less or no greater size were found in *Peromyscus leucopus* in Michigan and in New Mexico. Home ranges of *Peromyscus polionotus* on sparsely vegetated Santa Rosa Island, Florida, proved somewhat larger but averaged about ten acres in area. The home-range habit tends to restrict gene flow, because it restricts activity to a small area.

Dispersal of mutations through the population occurs principally when young mice leave the parental nest and wander in search of homes. The most complete study yet made of the distance of dispersal of *Peromyscus* was done by Howard (1949) on *Peromyscus maniculatus bairdii* in southern Michigan. By using nest boxes, Howard was able to mark 675 young mice while they were still with the parents, and he was able to follow subsequent movements by recovering the mice in nest boxes or in live traps. Maximum distance of dispersal was 3,300 feet. At least thirty per cent of the males and at least fifteen per cent of the females moved 550 feet or more before producing offspring. Howard estimated that at least four to ten per cent of 186 litters observed resulted from parent-offspring or intrasibship matings. Further study of the amount of inbreeding in *Peromyscus* populations should be made, for Howard's results have important implications. If seventy per cent of males and eighty-five per cent of females dispersed less than 550 feet before reproducing, a great amount of inbreeding is indicated. Inbreeding due to short distances of dispersal would be closer in these small populations than in populations made up of large numbers of individuals. Blair (1948) found that in the same area in which Howard worked average population densities of *bairdii* varied over a five-year period from zero on local areas to a maximum of 9.2 individuals per acre. Howard's (*loc. cit.*) data would indicate that the so-called hidden store of variability is possibly low in these mice. A mutation would tend to be exposed quickly to selective action of the environment. We do know, however, that recessive mutations are carried in wild populations of *Peromyscus,* and we know that stocks of *Peromyscus* generally fail to breed further after about five or six generations of intrasibship matings. The amount of hidden variability in *Peromyscus* populations, however, has yet to be determined.

TABLE 2. *Known distances of dispersal in three species of* Peromyscus.

Species	Locality	Method	Number of observations	Distance	Source of data
P. maniculatus bairdii	Michigan	Live trapping	4 ♀ ♀ 6 ♂ ♂	453 feet 1334 feet	Blair (1940)
P. maniculatus bairdii	Michigan	Nest box	78 ♀ ♀ 77 ♂ ♂	15% < 550 feet 31% < 550 feet	Howard (1949)
P. maniculatus blandus	New Mexico	Live trapping	3 ♀ ♀ 2 ♂ ♂	Up to ½ mile	Blair (1943b)
P. polionotus leucocephalus	Florida	Live trapping	14	1415±88.77 feet	Blair (1950)
P. leucopus noveboracensis	Michigan	Live trapping	154	⅔ > 100 yards	Burt (1940)
P. leucopus tornillo	New Mexico	Live trapping	3	Up to ½ mile	Blair (1943b)

Distances of movement of young *Peromyscus* of other species are known (table 2), but there is no certainty that these were complete dispersal movements, because the mice were not marked in the parental nest. Burt (1940) recorded movements of up to 900 yards by young *Peromyscus leucopus noveboracensis* in Michigan. Blair (1943b) recorded movements of between one-third and one-half mile by *Peromyscus maniculatus blandus* and of one-half mile by *Peromyscus leucopus tornillo*. Blair (1950) recorded a maximum movement of 1,970 feet by a young *Peromyscus polionotus leucocephalus* on Santa Rosa Island and found that fourteen young mice moved an average of 1,415 feet. These data indicate the general order of magnitude of dispersal distances in *Peromyscus*. More adequate data will be necessary before we can estimate the size of the effective breeding population (see Wright, 1943) with any degree of accuracy.

Uneven distribution within the species population is probably an important factor in geographic differentiation. The total population of the species is subdivided into many small, partially isolated populations. Under such conditions, the amount of both adaptive and non-adaptive differentiation depends on the amount of immigration per generation (Wright, 1943).

Uneven distribution within the range of the species results principally from: (1) preference of the species for a particular environment, (2) tolerance of other, marginal environments, and (3) more or less complete avoidance of other environments. Mapping the population density of a widely distributed population would show the species abundant in some places, scarce in others, and lacking from still others.

The pattern of distribution of any species of *Peromyscus* is so complex that no attempt has ever been made to analyze this pattern for an entire species. Much useful information is available, however, concerning the ecological distribution of some of the common species, and some important generalizations can be made regarding the possible role of isolation in geographic differentiation in this group. A few examples will suffice. The woodmouse (*Peromyscus leucopus*) is a semi-arboreal form that ranges over most of the deciduous forest region of the eastern United States. In this extensive region, this species is more or less continuously distributed over wide areas, or at least it probably was so distributed before the country was settled. Clearing of the land, with the retention of isolated woodlots,

has increased the amount of isolation of local populations and consequently has increased the opportunity for both adaptive and non-adaptive differentiation, for the species rarely occurs away from the forest environment in the eastern United States. Dice (1937a) found that in southern Michigan and in southern Indiana woodmice from woodlots as little as three miles apart differed significantly in shade of pelage color and in several body dimensions. No comparable samples from a continuously distributed population are available for comparison of the degree of local differentiation under the conditions of recent, man-made isolation and under conditions of continuous distribution. Cleared land is only a partial barrier to this species, for a few individuals do range into grasslands and fields in Michigan (Blair, 1940). If cleared land is abandoned and ecological succession continues, the barrier to dispersal of this species will gradually disappear.

The population density of the woodmouse may vary even where the forest is continuously distributed. In the Ozarks, the species is most common in the mesic forests of stream valleys and ravines, and it is generally scarce or lacking in the dry oak-hickory forest of the hillslopes. At Reelfoot Lake, Calhoun (1941) found the species mostly limited to areas not subject to inundation and mostly absent from lowland areas. In either case, the avoidance of certain types of forest should result in greater local differentiation than would result from isolation by distance alone.

The pattern of distribution of the woodmouse in the plains and prairies of the central United States is theoretically more favorable to differentiation than is the pattern in the eastern forest. The woodmouse is largely restricted to the wooded flood-plains of the streams in this predominantly grassland region. These flood-plain populations generally have linear rather than areal continuity. The range of each of these stream-valley populations is essentially one-dimensional in the sense that dispersal completely across the range may occur in a single generation. Linear continuity of distribution is much more favorable to differentiation than is areal continuity (Wright, 1943). The situation is complicated by the fact that the pattern of distribution is dendritic in type, following the stream pattern. It is further complicated by the fact that where the streams cross isolated forest areas, as in the Osage Hills of Oklahoma and the Cross-Timbers and Edwards Plateau of Texas, the distribution of the wood-mouse shows areal continuity, with the densest populations along the streams and less-dense populations in the scrub forests of the uplands. Measurements of the variation of wood-mouse populations along one of the many stream systems in the plains region and comparison with the variation under areal distribution would test the validity in nature of the theoretical differences in the amount of differentiation under these patterns of distribution. In northern Oklahoma, two samples of *leucopus* from tributaries of the same stream, about fifty miles apart airline and about twice that by way of the streams, differ by nearly seven millimeters in body length and show significant differences in foot length, femur length and in three of four skull measurements taken (author's unpublished data). Additional samples should be taken at lesser distances along the stream system.

In southern Texas and northern Tamaulipas, the wood-mouse shows areal continuity of distribution. This species is widely distributed in the semi-arid brush that blankets the coastal plain south of the Balcones fault, and distance is probably the principal isolating factor in this area. Differences in population density are evident even here. The population density appears to increase from north to south, and local variations in density occur apparently as the result of minor, local differences in density of the brush, in soil type, and in other features of the environment (author's unpublished notes). This does appear, however, to be the area

within the species range in which there is least subdivision into partially isolated local populations. The tendency to breed with neighbors will lead to some local differentiation even with such comparatively even distribution as this (see Wright, 1943).

The range of the wood-mouse extends westward in the southwestern deserts to central Arizona. In this region more than in any other part of its range, the species population is broken into numerous semi-isolated, local populations. The species preference for deciduous forest and brush environments is retained in this desert area with the result that strong ecological barriers to dispersal exist between widely scattered local areas of suitable environment. This is a pattern of distribution that should favor a great amount of local differentiation, and measurements of variation should be made in several of these local populations.

The pattern of distribution of the beach-mouse (*Peromyscus polionotus*) of the southeastern United States is complex even though the range of the species is comparatively small. This fossorial species is restricted to sandy soils. Along the eastern and northwestern coasts of Florida, the distribution of these mice is principally of the linear type. The mice are restricted to the narrow line of dunes fringing the beach, and they avoid the low, wet areas occupied by marsh vegetation or palmettos just behind the beach dunes. The linear continuity of the beach populations is frequently interrupted by bays and river mouths, with their associated unfavorable soils and vegetation. Inland in Florida, Georgia and Alabama, the distribution of the mice is more or less discontinuous, because the distribution of sandy soils is frequently interrupted by other, unfavorable soil types. The densest inland populations are found in cultivated fields, and the present distribution of these populations is closely correlated with human culture. Favorable patterns for differentiation are present in all parts of the range of this species.

The pattern of distribution of a species may be further complicated by the absence of the species from areas within the range that appear to be ecologically favorable. This is illustrated in *Peromyscus maniculatus*, the most widely distributed species of the genus. In the central plains this mouse is an inhabitant of grasslands, and from Oklahoma and the Texas Panhandle northward to Canada this species ranges completely across the plains. In eastern Texas, this species extends south through the tall-grass prairies of the coastal plain nearly to the Gulf coast. West of the Pecos River in Texas and New Mexico the species is common. It is apparently absent from the grasslands of most of western Texas with the result that there is no direct connection across Texas between the east-Texas and the Trans-Pecos populations. These populations are only 250 miles apart, yet a mutation that appeared in the Austin population would have to be transmitted over 800 miles, by way of the Oklahoma populations, in order to reach the Trans-Pecos population.

Geographic variation in most instances probably is influenced both by selection and by isolation. The opportunity for both agencies to act on the population is present in all species of *Peromyscus* investigated, and such an opportunity is probably present in all natural populations. In New Mexico, *Peromyscus maniculatus* with local linear distribution showed adaptive differentiation in the frequency of buff and gray genes on differently colored soils eighteen miles apart, but there was no differentiation on differently colored soils only four miles apart (Blair, 1947c). Populations twenty miles apart, on similarly colored soils and separated by a strong ecological barrier, showed no differentiation in the frequency of these color genes (table 3).

Eight species of rodents show adaptive dark pelage color on the Tularosa Malpais, a lava bed about forty miles long and from one to six miles in width. The Malpais population of *Peromyscus eremi-*

TABLE 3. *Relative effects of isolation by distance, ecological isolation and selection on local differentiation in frequency of pelage color alleles (buff, G: gray, g) in* Peromyscus maniculatus blandus. *Four stations in Tularosa Basin, New Mexico. Based on Blair (1947c)*

Stations compared				Distance between stations (miles)	Ecological barrier	Difference in gene frequency
Soil color	Frequency gray (g) %	Soil color	Frequency gray (g) %			
Dark red	43.24±3.85	Pinkish gray	45.49±5.10	4	None	Not significant
Dark red	43.24±3.85	Pinkish gray	75.13±2.37	18	None	Significant
Dark red	43.24±3.85	Pinkish gray	63.54±4.61	15	Strong	Significant
Pinkish gray	75.13±2.37	Pinkish gray	63.54±4.61	20	Strong	Not significant

cus, poorly isolated by ecological barriers, is more variable in pelage color than such species as *Peromyscus nasutus, Neotoma mexicana,* and *Neotoma albigula,* which are ecologically well isolated on the Malpais (Benson, 1933; Blair, 1943c). The dark coloration is evidently adaptive in all cases and natural selection favors survival of dark-colored individuals. The species populations with the highest proportions of well-adapted individuals are the populations with the greatest isolation on the Malpais, and the least adapted population is the one that is least well isolated.

Evidence for the Ecological Nature of Subspecies

Most species of *Peromyscus,* like many other species of animals, are subdivided into several geographic races or subspecies. Much of the recent work with *Peromyscus* has dealt with intraspecific, geographic variation, and this work has contributed materially to our knowledge of the nature of the subspecies. Geographic races, in *Peromyscus* at least, appear to be mainly the adaptive products of environmental selection, aided by partial isolation within the species population. Dice and Blossom (1937) regard the subspecies as an "ecological trend." A similar opinion is expressed by Mayr (1947) who holds that, "all geographical races are also ecological races, and all ecological races are also geographic races." Much of the evidence that the subspecies is principally an ecological phenomenon in *Peromyscus* has been previously stated (Dice, 1940a). A restatement of this evidence and some elaboration on it seems worthwhile.

Number of subspecies in relation to size and variability of area

Species with the greatest geographic ranges have the greatest number of geographic races, and the number of races is roughly proportional to the area occupied by the species. The deer-mouse (*Peromyscus maniculatus*), which ranges over most of North America from the Arctic Circle south to southern Mexico, has twenty-eight named geographic races, exclusive of insular forms. The woodmouse (*Peromyscus leucopus*), with a species range about one-half that of the deer-mouse, has twelve named, continental subspecies. The gopher-mouse (*Peromyscus floridanus*), restricted to peninsular Florida, is represented by a single race. The same relationship holds in the other species of *Peromyscus* (see table 5).

The range of a widely distributed population includes more different environments than does that of one with limited distribution. It is this fact rather than size of range that accounts for the proportional relationship between size of range and number of subspecies. This

TABLE 4. *Selected data showing trends in body dimensions in forest-inhabiting populations (first four entries) and grassland inhabiting populations (last four entries) of* Peromyscus maniculatus

Subspecies	Locality	Body dimensions, in millimeters				Source of data
		Body length	Tail length	Foot length	Ear length	
nubiterrae	Mountain Lake, Virginia	92.48±.41	89.65± .51	20.853±.070	17.858±.101	Author's data
gracilis	Cusino, Michigan	97.20±.82	88.28±1.06	21.700±.115	19.713±.118	Author's data
rubidus	Coos Head, Oregon	96.58±.52	92.65± .54	22.281±.079	18.662±.099	Dice (1941a)
rufinus	Cloudcroft, New Mexico	86.60±.37	70.25± .45	20.171±.065	18.376±.070	Dice (1944b)
bairdii	Lincoln, Nebraska	93.87±.48	54.66± .42	18.297±.061	14.951±.057	Dice (1941b)
ozarkiarum	Garnett, Oklahoma	92.60±.43	56.24± .43	19.071±.058	15.479±.071	Author's data
blandus	Marfa, Texas	99.37±.43	66.14± .55	21.208±.072	18.266±.061	Author's data
osgoodi	Miles City, Montana	95.10±.46	60.94± .41	20.137±.063	17.326±.077	Dice (1944a)

TABLE 5. *Relationships of the members of four cenospecies of the genus* Peromyscus

Cenospecies	Species	Number of continental subspecies	Number of insular subspecies
maniculatus	*maniculatus*	28	36
	polionotus	5	2
	melanotis	1	—
	sitkensis	—	3
	sejugis	—	1
leucopus	*leucopus*	12	3
	gossypinus	4	1
truei	*truei*	9	—
	nasutus	2	—
	comanche	1	—
	polius	1	—
	difficilis	3	—
	bullatus	1	—
boylii	*boylii*	10	2
	pectoralis	3	—
	oaxacensis	1	—
	hylocetes	1	—

is illustrated within *Peromyscus maniculatus*. Eastern North America between 35° and 55° north latitude is comparatively uniform in climate and vegetation, while the comparable region of western North America is marked by a diversity of environmental extremes. Only five subspecies of *maniculatus* are recognized by Osgood (1909) east of the ninety-eighth meridian and between 35° and 55° north latitude, while eleven subspecies are recognized in a comparable area west of the ninety-eighth meridian.

Distribution of morphological types in relation to vegetation types

Variations in body dimensions are often correlated, in *Peromyscus*, with major variations in the vegetational environment occupied (table 3). Two major morphological types have developed in *Peromyscus maniculatus*. One is a long-tailed,

large-eared, large-footed forest type. The other is a short-tailed, small-eared, small-footed grassland form. Each of these major types embraces several named subspecies, which are differentiated on pelage color and on minor variations in body and skull dimensions. In the east, the forest form (subspecies *nubiterrae, gracilis, abietorum, maniculatus*) ranges through the Appalachians from northern Georgia northward and extends on northward to Labrador. It ranges across the continent in the Canadian forests (subspecies *maniculatus* and *borealis*) and extends south through the Rocky Mountains and the mountains of the Pacific coast (subspecies *oreas, austerus, rubidus, gambeli, rufinus, artemisiae*). The grassland form (subspecies *bairdii, ozarkiarum, pallescens, nebrascensis, osgoodi*) occupies the grasslands of the interior of the continent and is surrounded on three sides by the long-tailed forest type.

In the eastern part of the continent, the grassland type is closely restricted to prairies, and the forest form is limited to forest environments. The ecological specificity of these two types is such that they apparently fail to interbreed even where they occur in the same area in Michigan (Dice, 1931; Hooper, 1942). Clearing of the forest has apparently favored the spread of the local grassland type, *Peromyscus maniculatus bairdii,* while the local long-tailed form, *Peromyscus maniculatus gracilis,* remains in favorable, uncleared forests. There seems to be no intergradation between the forest and grassland populations in the eastern part of the country, but the two are remotely connected by way of the western and Canadian populations.

In the western part of the continent, the distribution of the long-tailed and short-tailed forms is complicated by the irregular distribution of forest and grassland environments. Intergradation of these environments occurs commonly in this region in the sense that the grasslands of the plains and intermontane basins change through brush on the lower slopes of mountains to coniferous forests at higher elevations. In general, there is a correlated intergradation of body dimensions of *Peromyscus maniculatus.* The longest-tailed mice (*oreas, rubidus*) are found in the humid forests of the mountains and lowlands bordering the Pacific coast (see Osgood, 1909). The shortest-tailed forms (*osgoodi, blandus*) are found in grasslands. The *osgoodi* population inhabits semiarid grass or sagebrush-covered plains and foothills of Montana and neighboring states and provinces (Dice, 1944a). The *blandus* population is found in desert grasslands of intermontane basins from central Mexico to southern New Mexico. Populations showing all stages of intermediacy between the short-tailed and long-tailed extremes are found in the west, and there is considerable variation in tail length within the geographic limits of a single subspecies. Variations in body dimensions have been measured in samples of *Peromyscus maniculatus* populations from many more or less widely scattered localities in western North America (Sumner, 1918; Dice, 1938, 1939a, 1941a, 1944a).

Relationship between pelage color and color of background

The correlation between pelage colors of *Peromyscus,* and of other small mammals, and the color of the background soils has been discussed so extensively by recent authors that only a brief discussion is necessary here. Use of colorimetric methods, the Ives tint photometer (Sumner, 1927) and more recently the photoelectric reflectionmeter (Blair, 1947b), has provided fairly accurate measurement data for the comparison of pelage color and soil color. The strikingly obvious cases of color resemblance, on dark lava beds and on white sands, have received the most attention. Sumner (1926) pointed out the striking depigmentation of populations of *Peromyscus polionotus* occupying the more or less white sands of coastal dunes and islands in Florida. Inland populations, living on comparatively

dark-colored soils did not show this depigmentation. This case was later discussed by Blair (1944) in respect to what was by then known about the inheritance of the depigmentation character. Inland populations are homozygous for a recessive, major gene that produces "normal" pigmentation. The population on Santa Rosa Island, a white sand reef, is nearly or completely homozygous for the dominant allele, and it has a complex system of minor genes affecting the restriction of pigment. Populations of mainland beaches are more heterozygous than the island population, but the depigmentation genes predominate in these populations.

Desert mammals show many striking cases of color correlation with soil color. The colors of desert soils are little affected by the accumulation of humus, due to low rainfall, and in many places in our southwestern deserts dark-colored lava beds contrast strongly in color with the usually pale desert soils. Dice and Blossom (1937), in a classic study of the colors of desert mammals, called attention to the occurrence of several dark-colored races of *Peromyscus,* and of other small mammals, on lava beds. They also showed that populations living in mountain forests were darker in pelage color than populations of the same species living in nearby deserts. They also found that populations living on the lower slopes of the mountains were intermediate in pelage color. The color differences are attributed to selection on soils having, respectively, large, small, and intermediate amounts of humus.

Some species have developed dark-colored races on several different lava beds. The cactus-mouse (*Peromyscus eremicus*) is one example. In southern Arizona, California and northern Sonora, four of ten stocks of cactus-mice were from dark lava beds (Dice, 1939b). All of the stocks from lava beds averaged darker than the stocks from pale-colored desert soils, and two of the stocks were much darker than any of the others. In southern New Mexico, the species has similarly developed dark-colored races on at least two lava beds (Benson, 1933). Blair (1947d) analyzed color variations in five populations of *eremicus* from southern New Mexico. The dark-colored populations of the Tularosa Malpais and the Kenzin lava beds are separated by pale-colored populations on gray or pinkish-gray desert soils. A population of *eremicus* occupying a twenty-square-mile area of dark red soil averages darker in color than other populations eighteen miles away on pale, pinkish-gray soils.

Some of the lava beds, such as the Tularosa Malpais, have dark-colored populations representing several different species. Eight species of mammals show dark coloration on the Malpais (see Dice and Blossom, 1937; Benson, 1933; Bradt, 1932; Blair, 1943c). Subspecies names have been given to the local dark races of *Peromyscus nasutus, Neotoma albigula, Neotoma mexicana, Perognathus intermedius* and *Citellus variegatus.* The local race of *Peromyscus eremicus* has not been named because of its variability, which apparently results from the immigration of pale-colored individuals. Two other species, *Eutamias cinereicollis* (see Benson, 1933) and *Citellus interpres* (author's data) are known from the Malpais from a single dark-colored specimen each. The nearby White Sands contrast strikingly with the Tularosa Malpais in soil color. One species, *Perognathus apache,* is represented on the White Sands by a nearly white population (Dice, 1929). The deer-mouse (*Peromyscus maniculatus*) occurs sparingly on the White Sands, where it is similar in appearance to populations living on pale pinkish-gray desert soils (Blair, 1947c).

In a series of other studies, mostly on *Peromyscus maniculatus* and *Peromyscus leucopus,* Dice has shown that there is a general tendency for pelage color to be correlated with soil color. Such correlation is found even where the differences in soil color and in pelage color are comparatively slight to the human eye.

Zones of intergradation

Intergradation of morphological characters is the taxonomist's evidence that two dissimilar forms interbreed and consequently belong to the same species population. The term intergradation as used by the taxonomist has a variety of meanings. Character intergradation in geographically isolated populations, as used occasionally by Osgood (1909) and others, is hardly consistent with modern taxonomic concepts. Intergradation involves a geographic character gradient. Zones of intergradation may vary from wide to narrow. In the former case, the so-called zone of intergradation may be virtually the entire range of the species and may simply involve a character gradient connecting extremes at the opposite ends of the range. There is no meeting and interbreeding of two distinct forms; there is only progressive geographic change in the species population. Such cases usually occur where there is gradual change in environment from one extreme of the range to the other. Dice (1940d) analyzed such a case of intergradation in *Peromyscus maniculatus* in North Dakota after obtaining samples at ten stations along an east-west transect of the state. The environment changes gradually but not evenly from east to west across North Dakota in respect to elevation, aridity, soil color and vegetation. Dice found that there was a general increase from east to west in body size and in paleness of pelage color. There was no even transition in characters from east to west, for he found numerous local interruptions or reversals of the general trends. At no place could a line be drawn separating the eastern, dark-colored, smaller *bairdii* from the western, larger, paler *osgoodi*.

Sumner (1929a, 1929b) studied a narrow zone of intergradation between two races of *Peromyscus polionotus*. Intergradation in color and pattern characters of the pelage between the beach population of *albifrons* and the inland population of *polionotus* was found to take place in a strip parallel to the beach and extending inland for about forty miles. The comparatively abrupt break in the character gradient is apparently related to the abrupt change in soil color from the pale beach sands to dark-colored, inland soils. The zone of intergradation is actually on inland soils, back of the narrow strip of pale-colored beach soils. This zone of intergradation is an area into which are dispersed and intermixed the very different color types that occur on each side of it (Blair, 1944).

Dice (1941b) studied the intergradation between *Peromyscus maniculatus nebrascensis* of the Nebraska sand hills and *Peromyscus maniculatus bairdii* of the prairies adjacent to the sand hills. The sand-hills soils are generally much paler than the soils of the surrounding regions. The mice of the sand hills are predominantly pale in color, but every local population has a high degree of variability in shade of color. A darker-colored population surrounds the sand hills on all sides. Dice attributes the high variability of the sand-hills population to constant interbreeding with the surrounding, darker-colored population. A general east-west cline in body dimensions passes through the sand hills without a steepening of the character gradient.

Factors in Speciation of Peromyscus

Speciation in the sense of the splitting off of a new species, as opposed to evolutionary change, with time, in an old species, involves isolation of a part of the previously interbreeding population. The question of how this isolation arises and of how isolation fits into the sequence of speciational changes has been much debated in recent years. The various conflicting theories concerning the origin of this important speciational step have been ably reviewed by Mayr (1947) who has presented the case for geographical as opposed to the various theories of so-called sympatric isolation.

Spatial isolation

The evidence from *Peromyscus* indicates that, in this group at least, geographic discontinuity is generally, if not always, the first step in the splitting off of a new species. The evidence is mostly circumstantial, since we are limited to observation of the present relationships of the members of a species group. The evidence for geographic speciation may be summed up in two statements: (1) "offspring" species are found around the periphery of the range of the "parent" species, or they are found in the southwestern mountain and desert region, where, in isolated mountain ranges there is opportunity for what Mayr (1947) calls macrogeographical isolation, (2) different species within the same cenospecies generally have rather similar ecological preferences.

Four cenospecies of *Peromyscus,* with two or more species each, are sufficiently well known to give evidence of the course of speciation in this group. In each cenospecies, one species has a much larger geographic range and has undergone more subspeciation than the other species (table 5). This largest species, with one exception in the *truei* group, is presumably ancestral to the other species of the same cenospecies. The species *maniculatus,* with a distribution over most of North America, has apparently given rise to four other species. Each of these derivative species is located on the periphery of the range of *maniculatus.* The species *polionotus* occurs in Florida, Georgia, South Carolina and Alabama, and it is separated by about 100 miles from the nearest known population of *maniculatus.* This nearest population of *maniculatus* belongs to a long-tailed, forest-inhabiting race, and *polionotus* seems to have originated instead from the short-tailed, prairie-inhabiting populations. The subspecies *pallescens* of the Texas coastal prairies is the nearest geographically of these prairie forms and is the most similar morphologically to *polionotus* of any race of *maniculatus* (Osgood, 1909, p. 105). A gap of over 500 miles now separates the range of the Texas mice from that of *polionotus.* The present geographic relationships of these two species can be explained if we assume continuous distribution of *maniculatus* across the coastal plain in Pleistocene time. This distribution possibly, but not necessarily, might have been only in a narrow strip along the Gulf beaches. With encroachment of the Gulf on the land during Pleistocene inter-glacial stages, there was the opportunity for a part of this population to be isolated in Florida, for parts of the present land area of Florida projected as islands during these periods (see Cooke, 1939). The postulated coastal-plain population of *maniculatus* disappeared eastward of Texas, effectively isolating the Florida population. An ecological barrier now separates the two coastal-plain populations, for the forests and coastal marshlands now occupying the coastal plain eastward from eastern Texas are avoided by both species.

The *polionotus* population has now diverged from *maniculatus* to the extent that sexual isolation would prevent interbreeding if the two populations should come to have overlapping ranges (Blair and Howard, 1944). The *polionotus* mice have also differentiated from the *maniculatus* in social behavior (*idem*). They differ morphologically in the presence of the previously mentioned complex of genes affecting distribution of pigment over the body. They differ only slightly in size and body proportions from *Peromyscus maniculatus pallescens.* They have differentiated genetically from *maniculatus* to the extent that some F_1 male hybrids are sterile, while all female and some male hybrids are fertile (Watson, 1942; author's unpublished data).

Another species of the *maniculatus* group is *sitkensis,* which occurs on coastal islands off the coast of Alaska and British Columbia. Again the derivative species is on the periphery of the range of the parent species, and again insular ori-

gin is strongly suggested. The present distribution of this species is somewhat puzzling. The population has not spread to the mainland, but the species occurs on several coastal islands, while *maniculatus* occurs on others. This species is known only from museum materials, so the degree of differentiation from *maniculatus* is unknown. The *sitkensis* populations are poorly differentiated morphologically from the populations of *maniculatus* occupying the nearby mainland (see McCabe and Cowan, 1945, p. 194). The *sitkensis* populations will have to be tested against *maniculatus* for intrinsic isolating mechanisms before we can be sure of their stage of speciation.

A third named species of the *maniculatus* group is *sejugis,* which was described by Burt (1932) from Santa Cruz and San Diego Islands in the Gulf of California. This mouse is described as being larger than the race of *maniculatus* occupying Baja California and as showing minor differences in skull characters. The stage of speciation of the *sejugis* populations remains to be determined.

A fourth named species apparently derived from *maniculatus* is *melanotis,* which occurs at high elevations in various mountain ranges of Mexico. According to Osgood (1909), *maniculatus* occurs principally at lower elevations and is replaced by *melanotis* in the mountains, although the two are said to occur together in some places. Little is known about the degree of differentiation of *melanotis* from *maniculatus,* as it is known only from museum materials. Osgood (*op. cit.*) indicates that there is well-marked morphological differentiation. Mating preference tests are needed to show the degree of isolation from *maniculatus.*

Geographic relationships between the two species of the *leucopus* group are somewhat the same as those between *maniculatus* and *polionotus.* The species of *leucopus* is widely distributed, ranging from southern Mexico to Nova Scotia. It is replaced by the species *gossypinus* on the Gulf coastal plain from eastern Texas and Oklahoma to the Atlantic, and in Florida. The two species occur together in some areas (Dice, 1940c; Calhoun, 1941) and apparently fail to interbreed. Again the derivative species is on the periphery of the range, and again it is in the southeastern states. The species *gossypinus* may have originated under the same conditions of isolation under which *polionotus* presumably originated. In the present case, one or both of the two related species populations have spread until there is an overlap of range. Psychological barriers to interbreeding must have developed during the period of geographic isolation to maintain the present discreteness of the populations, as the two species are interfertile and will cross in the laboratory when there is no choice of mates (Dice, 1937b). The principal morphological differences between the two species are size differences. Reoccupation of intervening territory by the once geographically isolated populations has been possible because there is no intervening ecological barrier. Both of these species are inhabitants of deciduous forests, so the same forests that act as a barrier to reinvasion of territory by *maniculatus* and *polionotus* serve as a dispersal route for these species. The *gossypinus* population seems to have diverged less from *leucopus* than *polionotus* has deviated from *maniculatus,* for morphological differences are less evident, and there is no apparent infertility in F_1 hybrids between *gossypinus* and *leucopus* (Dice, 1937b).

In most of the foregoing cases, there has been the strong possibility that speciation began with isolation on coastal islands. Numerous insular populations of *Peromyscus,* treated taxonomically as subspecies, are presumably in incipient stages of speciation. Six of the seventeen species in the four cenospecies treated here have insular subspecies, and the species *maniculatus* has no less than thirty-six named insular subspecies (table 5). None of these insular populations has been tested against mainland or other insular

populations to determine whether or not intrinsic isolating mechanisms have been developed. It is quite possible that when such tests are made some of these populations on coastal islands will satisfy the biological criteria of species.

The *truei* group provides a case in which speciation has taken place in the continental interior, but still at the periphery of the species range. The species *nasutus* ranges widely in the mountains of New Mexico, where it is largely limited to the pinyon—cedar and other forest communities. A related species, *comanche,* apparently derived from *nasutus,* occupies the cedar forest of deep canyons along the escarpment of the high plains in the Texas Panhandle (Blair, 1943d). The two populations are separated by over 100 miles of barren, short-grass plains, which constitute an effective ecological barrier to dispersal of both species. The two populations must have had a continuous distribution across the plains in the past. This continuous distribution may have existed as recently as late Wisconsin time, when a southward shifting of climatic belts caused Lake Bonneville to be filled to the Stansbury strandline. Post-Pluvial increase in aridity in the high plains, leading to present environmental conditions in the region, would account for the elimination of the population from the plains and for the isolation of a segment of the population along the escarpment of the plains. If we follow estimated Pleistocene chronology (Flint, 1947), the isolation of *comanche* from *nasutus* presumably occurred less than 10,000 years ago. The *comanche* population is morphologically well differentiated from *nasutus,* and some F_1 male hybrids between the two species are sterile (Blair, 1943d).

The *truei* group has undergone additional speciation. The species *truei* has the widest distribution of any member, ranging from Oaxaca in Mexico north through the mountains to Colorado and Oregon, and the other species are possibly derived from this population. The species *nasutus,* the probable ancestor of *comanche,* ranges through the mountains of Colorado, New Mexico and eastern Arizona. Three other named species, *polius, difficilis* and *bullatus,* occurring on mountains in Mexico, are assigned to the *truei* group by Osgood (1909). Little is known about these forms. If they are good species (i.e., reproductively isolated) then four related species with lesser ranges, and possibly more recent origins, occur within the range of *truei.* The relationships between the *truei* and *nasutus* populations are fairly well known in New Mexico. There is some vertical separation of the two populations, with *truei* generally occurring higher in the mountains than *nasutus,* but both occur together in many places (Dice, 1942). Blair (1943c) found both species ranging down to the base of the Sacramento Mountains in the Tularosa Basin, but there is a tendency for *truei* to predominate at higher elevations and for *nasutus* to be most numerous at lower elevations. Sexual isolation prevents interbreeding of these species (author's unpublished data). These populations have differentiated to the extent that male hybrids are sterile, while females are not (Dice and Liebe, 1937). There is well-marked morphological differentiation. This is a case that might be used to support the thesis that ecological isolation in different montane vegetation belts is the initial isolating mechanism. An equally good, if not better, explanation is that *nasutus* and the other derivative species originally differentiated under conditions of macrogeographical isolation in isolated mountain ranges, of which there are many in Mexico and the southwestern United States (see also Dice, 1940b). Once they had developed intrinsic isolating mechanisms, these species would maintain their distinctness as they later spread back into the mountain ranges occupied by the parent species. It is not unlikely that in this region, which offers many opportunities for macrogeographical isolation, some isolated populations do spread before de-

veloping isolating mechanisms and are consequently swamped by the parent population. The present vertical zonation of closely related species in mountain ranges might possibly result from the ecological conditions that pertained in the mountain regions in which the species were originally isolated and differentiated. A species such as *nasutus,* which prefers the vegetative complex of intermediate elevations, may have originated by isolation in a comparatively low mountain range, where it developed a preference for, and became adapted to, pinyon and oak forests. Once the species succeeded in spreading to other, higher mountains, it probably would still retain its preference for such environments, and we would have the type of vertical zonation that we see in *nasutus* and *truei.* If *comanche* should ever spread back into the range of *nasutus,* these species would probably show similar zonation, with *comanche* in the cedars of the lowest elevations being replaced by *nasutus* in the pinyons of higher elevations.

The *boylii* group probably has had a speciational history similar to that of the *truei* group. This group is also limited mostly to the mountains of the southwestern part of the continent. The species *boylii* ranges from Guatemala north to Oregon and Utah, and it extends eastward to the Ozarks. The species *pectoralis* ranges from Michoacan in Mexico to the Edwards Plateau in Texas. The range of *pectoralis,* therefore, lies almost entirely within the range of *boylii.* As in the *truei* group, the present distribution may represent the results of isolation of a population in one of the many mountain ranges of the region until isolating mechanisms developed, followed by a subsequent range extension by the new species. Both species are closely associated with forested rock bluffs, and discontinuous distribution of such habitats is very favorable for isolation of populations. Two other species of the *boylii* group have been described from Mexico. These are little known, and they may be geographic races of *boylii* (see Osgood, 1909, p. 159).

The significance of peripheral populations in speciation in the avian family Dicruridae has been discussed recently by Mayr and Vaurie (1948). They concluded that isolation, either on islands or along the periphery of the range, is a powerful stimulus toward the development of aberrant populations. In combination, the two factors are particularly potent.

Ecological isolation

Invasion of a new environment by the parent species is hypothesized by some workers (see Thorpe, 1945) as the original cause of discontinuity in the population and, consequently, as the initial event in speciation. The evidence from *Peromyscus* offers no support for this theory. The habitat preferences among related species in the groups discussed above are generally similar. The species *polionotus* and the subspecies *Peromyscus maniculatus pallescens,* from which *polionotus* apparently originated, are both grassland inhabitants. There are obvious ecological differences between the grasslands of the Texas coastal prairie, where *pallescens* occurs, and the grasslands occupied by *polionotus* in Florida, but these habitats are far more similar than the environments occupied by different geographical races of *Peromyscus maniculatus.* The coniferous and deciduous forests inhabited by such geographical races of *maniculatus* as *oreas, gracilis, nubiterrae* and others are extremely different habitats from the grasslands occupied by such races as *bairdii, pallescens* and *nebrascensis,* but these forms have not evolved into new species.

No marked ecological change has accompanied speciation in the *leucopus* group. The species *leucopus* and the species *gossypinus* are both inhabitants of deciduous forests. The former prefers upland forests in some, but not all, parts of its range, while the latter prefers moist, lowland forests, but the two do occur to-

gether in some places (Dice, 1940c; Calhoun, 1941). The members of the *truei* group are all inhabitants of forests in southwestern North America. The incomplete zonation of the species of this group in different montane forest belts might support the case for ecological or microgeographical isolation, but an alternative hypothesis has been presented earlier in this discussion. The species *boylii* and *pectoralis* of the *boylii* group show the least habitat differentiation of any species. Both are restricted to forested or brush-covered rock bluffs and talus slopes. The two species occur together at various places on the Edwards Plateau, where they are said to have similar habits and habitats (Bailey, 1905).

Several cases of two subspecies of *Peromyscus maniculatus* occurring in the same region have been reported. Since such cases might be used to support the argument for microgeographical isolation, it is profitable to review what is known about the relationships of these populations. Osgood (1909) reported both *osgoodi* and *artemisiae* from the same area in Glacier Park, *oreas* and *austerus* from the coast of Washington, and *algidus* and *borealis* (= *arcticus* of Osgood) from the same area in northwestern Canada. Murie (1933) found the range of *osgoodi,* a grassland form, and *artemisiae,* a forest inhabitant, meeting in Glacier Park. He reported that the two races lived together in cabins at the edge of the forest without interbreeding, and he attributed the failure of these populations to interbreed to psychological factors. These mice should be tested in the laboratory for the presence of isolating mechanisms. One might argue that this is a case in support of microgeographical or habitat isolation, but an alternative, and to me more reasonable, explanation is that this is a case of secondary contact between the grassland and forest populations. (See also Mayr, 1942, fig. 22.)

Another case of two subspecies in the same area is almost certainly a case of secondary contact between populations. The races *gracilis,* a forest inhabitant, and *bairdii,* a prairie form, overlap in range in the upper and lower peninsulas of Michigan (Dice, 1931; Hooper, 1942) and in New York state (Hamilton, 1950). The evidence indicates that the present overlap of range has occurred largely as a result of the clearing of the forest and a subsequent range extension by *bairdii.* Hooper attributes his failure to find intergrades between the two races in museum materials to ecological separation, with *gracilis* in the forests and *bairdii* in cleared lands. This case justifies further investigation. No critical study has been made to determine whether there is actual contact of the two populations, as in the Glacier Park populations, or to show the behavior of the two if there is contact. The populations should be tested in the laboratory for intrinsic isolating mechanisms. If sexual isolation does exist between two populations in the same region that are still connected remotely by chains of other races, as in the cases mentioned above, a different sequence of speciational events is involved than when isolation originates from the simple, spatial isolation of a fragment of the population. If, as Murie (1933) believes, *osgoodi* and *artemisiae* are sexually isolated, the development of isolating mechanisms apparently has not been preceded by a complete discontinuity in distribution. The mechanisms of reproductive isolation would have arisen in this case due simply to isolation by distance. A major break in the continuity of the interconnecting populations would result immediately in two separate-breeding populations that would satisfy most modern definitions of species. Macrogeographical isolation is a necessary step in this case of speciation just as in the simpler case of original spatial discontinuity discussed earlier, but the sequence of speciational events is different.

Course of Speciation

Two possible courses of speciation are indicated in *Peromyscus* and geographic discontinuity is required in both. In the

first case, the one for which there is the best evidence, geographic discontinuity at the periphery of the species range, on islands, or on isolated mountain ranges is the first step toward speciation. Once the two populations are prevented from interchanging genes by spatial isolation they will undergo both adaptive and non-adaptive differentiation in morphological, physiological and psychological characters. Genetic differences will eventually become so great that interchange of genetic materials is no longer possible because of hybrid sterility. The most important step in this differentiation is, however, the development of intrinsic isolating mechanisms that would prevent interbreeding of the two populations. Once this stage has been reached the two populations can occupy the same territory without losing their distinctness through interbreeding. The course of speciation outlined here is one that has been emphasized in recent years (Dobzhansky, 1937, p. 256; Mayr, 1942, p. 154; Mayr, 1949 and others). My interpretation of the evidence from *Peromyscus* is that this course of speciation certainly is taken in this group and that it is probably the principal way in which new species originate in this genus.

A second possible course of speciation in *Peromyscus* is one in which genetic divergence precedes complete geographic discontinuity of the speciating populations. In this case, populations at extreme ends of the species range may differentiate due to isolation by distance, and these populations may differentiate in ecological preference to the extent that they may come to occupy the same region but different environments. The two extreme populations are held together in evolution by the intervening populations, however, and there is no proof that, barring outside interference, there would ever be breeding discontinuity between the two. Extinction of interconnecting populations would, of course, break the continuity of the two end populations. Cases of opposite ends of chains of races in the same region are numerous in the literature (Mayr, 1942, p. 180). The several similar cases in *Peromyscus maniculatus,* mentioned earlier above, indicate the possibility of such a course of speciation in this group, but any convincing proof is lacking.

The marked geographic variations in morphological characters and in ecological preferences in such species as *Peromyscus maniculatus* seem, on the basis of our evidence, to be of little or no significance to the splitting off of a new species. A new species can originate without the parent population having undergone marked geographic differentiation. There is no evidence that, in mammals, a population can be split into separate breeding units except by outside events. Speciation seems to be the fortuitous result of major distributional changes that result, in turn, from major regional changes in environment. Geographically differentiated sub-populations within the species range, the subspecies of the taxonomist, cannot be regarded, therefore, as incipient species, although extinction of interconnecting races, as by regional, environmental change, would result in the immediate production of two species from two subspecies if intrinsic isolating mechanisms had already developed in the end populations. A similar opinion of the unimportant role of geographic races in speciation is held by Wright (1949, p. 386).

Geographic variation is of considerable evolutionary importance to the species even though geographic races rarely appear to be involved in speciation. Wright (1931, 1949, and other papers) has pointed out the evolutionary value to the species of the many intraspecific genetic complexes that result from partial isolation within, and local selection on, the species population. The consequent increase in genetic variability of the species frees evolution from dependence on rare favorable mutations, and favors rapid evolution, but not splitting, of the species. We might add that wide distribution and wide variation in ecological preference

favor survival of the species. The species *maniculatus* occurs in so many environments in North America that no foreseeable environmental change short of inundation of the continent would probably eliminate all local populations. The species *floridanus,* restricted to a single type of environment in Florida, could be eliminated by a climatic change that eliminated this environment. The main advantage of a species wide genetic and ecologic variability to speciation is that, because of their wide distribution, such species have the most opportunities to be split by external factors into separate-breeding populations.

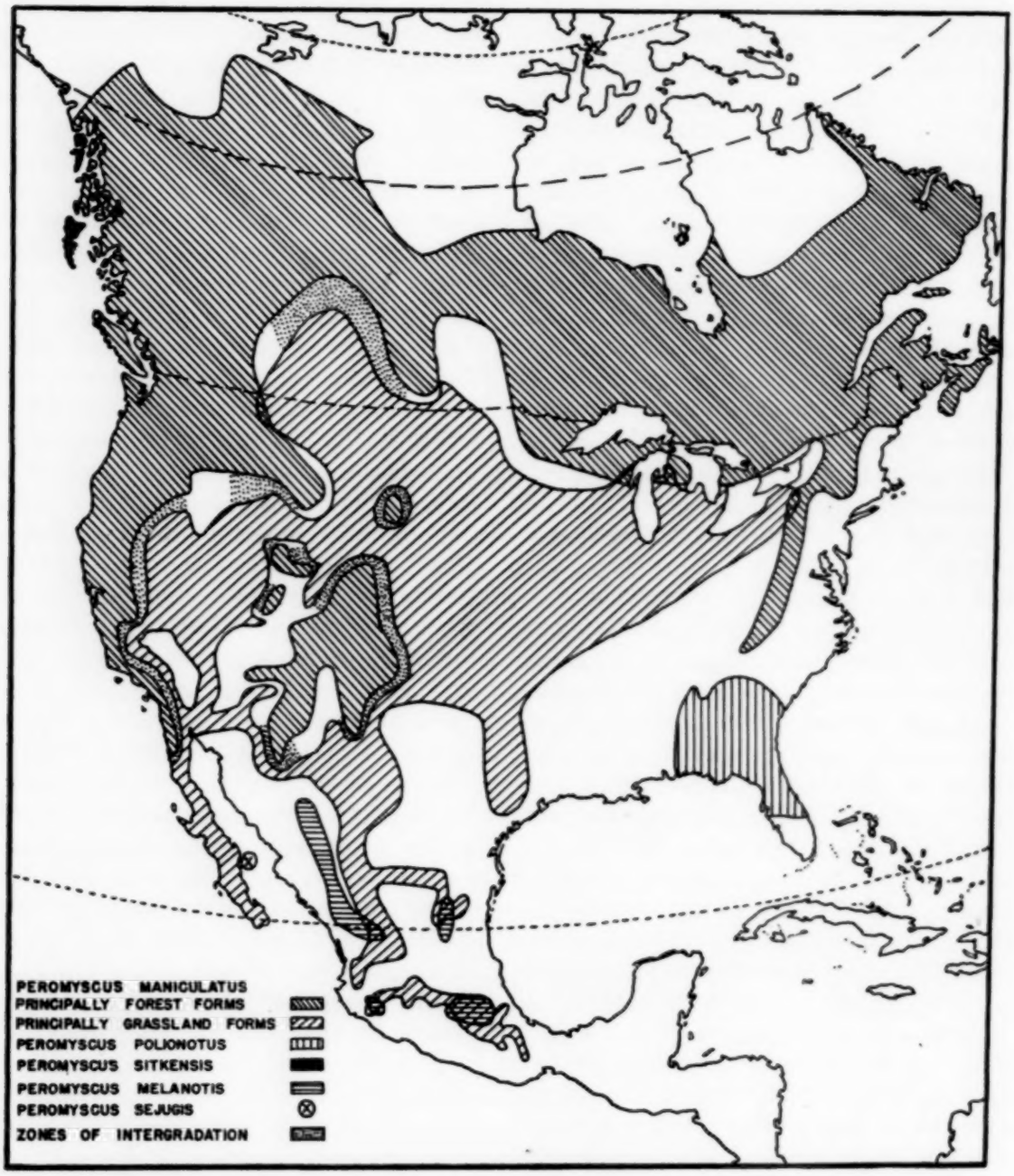

FIG. 1. Distribution of the five species of the cenospecies *Peromyscus maniculatus,* showing peripheral position of the species presumably derived from the species *maniculatus.* Approximate distribution of principally grassland-inhabiting and principally forest-inhabiting populations of *Peromyscus maniculatus.* Based on Osgood (1909) and later sources.

Summary

Geographic variation in mice of the genus *Peromyscus* is attributable to mutations, selection by the environment, and reduced gene flow through partial isolation of large or small segments of the species population. Wild genotypes are difficult of analysis in this group, but some mendelian alleles having important effects on wild populations have been described. Selection by the physical and biological environment is probably the most important agency determining the characteristics of sub-populations within the species range. There is evidence that simple predation is a selective agency of major importance in this group. Partial isolation of sub-populations because of ecological barriers and because of isolation by distance is another important factor in geographic variation. The home-range habit tends to restrict gene flow; dispersal of young on reaching sexual maturity has the opposite effect. The species is always unevenly distributed in its range due to preferences for certain environments, tolerance of others and avoidance of still others. The pattern of distribution varies within the species range, and in some parts of the range it may be more favorable to local differentiation due to isolation than it is in others.

Geographic races are ecological phenomena. There is no difference between ecological races, geographic races and subspecies. Species with the greatest geographic ranges, occupying many environments, have the greatest number of geographic races, and the number of races is roughly proportional to the area occupied by the species. There is a correlation, in some species, between certain body dimensions and the vegetational environments occupied. There is a general correlation between pelage color of *Peromyscus* and the color of the soils on which the mice live. The width of zones of intergradation between geographic races is related to the rate of geographic change in environment.

The present distribution and biological relations in four cenospecies of *Peromyscus* indicate that speciation is commonly initiated in this group by the simple geographic isolation of a segment of the population. There is evidence that new species have originated at the periphery of the range, on coastal islands, and probably on isolated mountain ranges. No marked change in habitat preference has accompanied speciation in *Peromyscus,* and it is unlikely that invasion of a new environment would have sufficient isolating effect to permit speciation. Opposite ends of chains of races have differentiated morphologically and in habitat preference, and intrinsic isolating mechanisms have possibly developed between some of these end populations. Extinction of intermediate populations would complete speciation in this case. There is no evidence that speciation would go to completion (i.e., breeding discontinuity) without this outside intervention.

Geographic variation is favorable to the survival and evolution of the species, without the splitting off of new species. Wide distribution of the species population is favorable for speciation, because the most widely distributed population will have the most opportunities to be split by external factors into separate-breeding populations.

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POLYMORPHISM IN A WOODPECKER (*PICUS FLAVINUCHA*)

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Picus flavinucha is a foothill species found along the Himalayas from Mussoorie throughout Assam, Burma and on to Siam and Indochina as far east as southern China and south to Malaya and Sumatra. There are currently ten subspecies recognized throughout that area. In all cases these forms differ from the typical form, *flavinucha* found from Nepal east through Assam and Burma to the Shan States, by size and variations in color.

If the typical race *flavinucha* is taken as a standard, then its particular size measurements may be assumed to be the character *S*, and its color, a rather pale shade of yellow and green, as *Y*. Its lack of any distinctive suffusion of melanins or phaeomelanins may be characterized as *p*. It should be understood here that these letter symbols are merely used to express the phenotypes. I have no genetic basis for assuming that these are merely single gene differences. Ranging the forms from west to east and south geographically, we then have the following:

kumaonensis	*S* +	*Y* +	*p* [1]
flavinucha	*S*	*Y*	*p*
archon	*s*	*Y*	*p*
lylei	*s*	*Y*	*p*
pierrei	*s*	*Y*	*p*
styani	*S*	*y*	*P*
ricketti	*S*	*y*	*P*
wrayi	*s*	*y*	*P*
mystacalis	*s*	*y*	*P* +
korinchi	*s*	*y*	*P* +

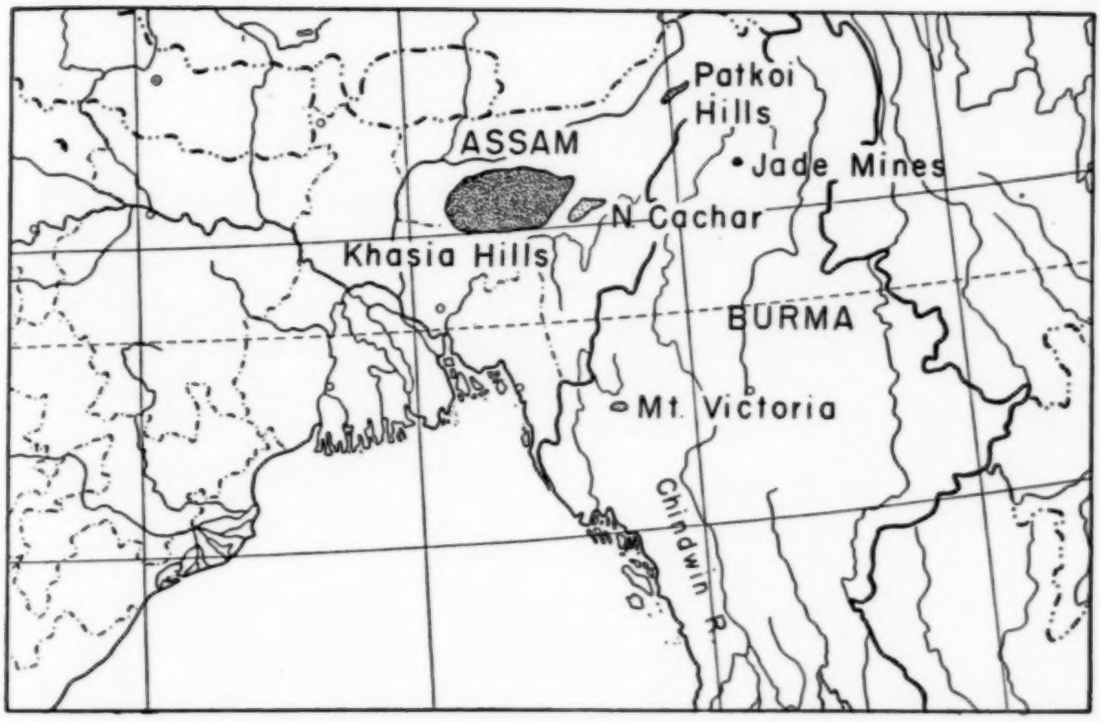
As might be expected the populations immediately adjacent to *flavinucha* geographically, differ in only one character, or in a heightening of two characters (as in *kumaonensis*), while populations at a considerable distance (as *mystacalis* from Sumatra) differ in all three characters. There are continuous clines throughout the total area in *S*, and discontinuous clines in *Y* and *p*.

The range of typical *flavinucha* is the largest geographically of any of the races and encompasses a number of different ecological zones. One of the most significant of these is the zone of heavy rainfall accompanying dense forest along the axis of the Khasia Hills, N. Cachar and the Naga Hills and Patkoi Hills on the Assam-Burma border. The area appears to be significant because it is isolated geologically and ecologically, and much speciation has occurred there (see fig.).

Examination of specimens of *Picus flavinucha* from this general area shows at once that a new color character is emerging. This is a sort of saturation, a deepening of the yellowish tones towards orange, and the appearance of an olive wash over the lower surface of the specimens. There is no accompanying size difference, but at first sight examination of birds from this northern Assam area indicates a valid subspecies with a definite geographical range which could be characterized as *S y p* +, one good color character being present and a heightening effect in a second.

Further examination has revealed that this combination is in fact a polymorphism, which crops up, apparently independent of geographical or other factors, in the general area. It is possibly an incipient speciation stage. At the present level of inquiry I can find no particular adaptive significance to these color characters although there is a tendency to correlation of increase in color saturation with increased humidity. However, the correlation is by no means complete in

[1] The characters of this race, larger size and paler color, represent a heightening merely of *S* and *Y*, and in order to express this I have added a plus sign to the letter.



Map of Assam and Burma, an area inhabited by *Picus flavinucha flavinucha*. In the two dark-shaded areas, the Khasia Hills and the Jade Mines, the saturated color form, *S y p +* occurs. In the light shaded areas, N. Cachar, Patkoi Hills, some specimens show the character, others do not. Typical *flavinucha* occurs in the intervening and unshaded parts of the map.

this case. As the accompanying map indicates, adjacent areas either show a cline in the direction of this character, *S y p +*, or else no indication of it. It would be most valuable to develop an explanation for the evolution of selection pressure leading towards the adoption of such apparently non-adaptive characters.